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ORAL PRESENTATIONS' ABSTRACTS

OP-001

Sustainable Biomaterials of bacterial origin and their use in Biomedical Applications

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INTRODUCTION: There is a huge need to find replacements for petrochemical-derived plastics which are not sustainable, degradable and lead to high concentrations of recalcitrant plastics in the soil and the sea. In the medical arena, there is not much attention paid to the sustainability of the materials used. In this work we have focused on the production and use of bacteria-derived sustainable biomaterials for use in biomedical applications.

Two main types of biomaterials have been focused on, including polyhydroxyalkanoates, PHAs,(1) and bacterial cellulose, BC, (2). PHAs are polyesters produced by a range of bacteria that are biodegradable in the soil and sea and are also resorbable in the human body and highly biocompatible. BC is also produced by a range of bacteria, is a sustainable green polymer, degradable in the soil, highly biocompatible. Hence, both PHAs and BC can be used for biomedical applications.

METHODS: Poly(3-hydroxybutyrate), P(3HB), a stiff scl-PHA was produced using Burkholderia sp and an elastomeric mcl-PHA was produced using Pseudomonas sp., at 30L scale using the fed batch mode. The polymers were characterised with respect to mechanical and thermal properties and processed using gyro spinning, electrospinning and Fused deposition modelling (FDM), melt electrowriting and dip moulding. The constructs were subjected to in vitro and in vivo biocompatibility analysis. Bacterial cellulose was produced under static culture conditions using G. xylinus.

RESULTS: P(3HB), its blends and composites were used for bone tissue engineering, drug delivery, coronary artery stents and nerve tissue repair. The mcl-PHA was used for the development of cardiac patches, nerve guidance conduits, wound healing patch, bioartificial pancreas and bioartificial kidney. In all cases the results were extremely positive both from a mechanical and functional context, in vitro and in vivo. The BC was a highly nano-fibrillated and was surface modified to create antibacterial bacterial cellulose. BC was also used as a filler for P(3HB) based composites.

DISCUSSION & CONCLUSIONS: In conclusion, we successfully produced and used bacteria-derived sustainable biomaterials for a variety of biomedical applications, including hard and soft tissue engineering. Both PHAs and bacterial cellulose have a lot of potential in the future as sustainable materials of choice.

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Keywords: Biomaterials, Composite materials

OP-002

Fibrin and silk fibroin as complementary materials of biocomposites for tissue engineering

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INTRODUCTION: In the context of tissue engineering, a single material often fall short of meeting all the necessary requirements to obtain a fully functional substitute for native tissue [1]. For instance, fibrin is known as a natural polymer with an excellent bioactivity, versatility and processability [2], but restricted in the biomedical applications by its cell-mediated contraction and insufficient mechanical strength [3]. In this work, we overcome these drawbacks by combining fibrin with another extraordinary natural polymer, silk fibroin, with a simple and reproducible biofabrication approach [4].

METHODS: The fabrication of fibrin and silk fibroin composites was carried out by injection-molding. The internal structure of the resulting scaffolds was investigated by scanning electron microscopy. The mechanical properties were characterized by rheology and burst strength. The fibrin/silk fibroin scaffolds were seeded with primary cells, such as human venous endothelial cells (HUVECs) and human arterial smooth muscle cells (HUASMCs), to test respectively the bioactivity and the resistance to the cell-mediated contraction. Confocal microscopy was used to analyze the cell adhesion and morphology. The feasibility to fabricate composite scaffolds of a tubular shape was also explored.

RESULTS: Homogenous, porous and reproducible composite scaffolds of fibrin/ silk fibroin were obtained by adopting injection-molding approach. The developed composites featured significantly higher mechanical properties than those made of only fibrin. The cellular studies showed a formation of confluent and adherent endothelial cell layer and minimal cell-mediated contraction. We demonstrate the feasibility to fabricate composite scaffold with a tubular shape, which could be bended 180 ° without kinking, speaking for outstanding flexibility.

DISCUSSION & CONCLUSIONS: With a simple and scalable biofabrication approach, we obtained composite scaffolds, characterized by synergy between complementary components, i.e., fibrin's exceptional bioactivity was combined with the mechanical stability offered by silk fibroin. The flexibility together with the versatility to mold into different shapes make the developed composite scaffolds promising candidates for cardiovascular tissue engineering applications.

ACKNOWLEDGEMENTS: The authors acknowledge the NanoMatFutur Program of the German Ministry of Education and Research (BMBF, grant number 13XP5136).

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Keywords: Composite materials,

OP-003

Growth Factor delivery through electrospun microfibres for tendon regeneration

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INTRODUCTION: Proliferation and differentiation towards particular lineages can be regulated by signalling from cells and the niche they inhabit. For example, Mesenchymal Stromal Cell (MSCs) fate is influenced by soluble biochemical factors, including growth factors, and also by biophysical cues provided by co-localised extracellular matrix arrangement and surrounding mechanical forces. To address the inherent limitations of supra-physiological dosage administration to drive specific phenotypes and derive optimal release kinetics, biomaterial-based approaches have emerged as a powerful tool. These approaches can be tailored to modulate proliferation, self-renewal, and tenogenesis of MSCs, drawing on the synergy between stimuli that incorporate a combination of molecules, such as growth factors, released in a controlled manner in a three-dimensional mechanically supportive scaffold.

METHODS: Three different growth factors (GDF5/6/7) have been analysed individually to assess their tenoinductive potential on MSCs. The synergic effect of growth factors and physoxia are investigated as major factors that recapitulate the tendon developmental processes. Tenomodulin immunofluorescence and RT-qPCR techniques have been performed in parallel on hBM-MSCs and TSCs, to assess the expression of tendon-linked transcripts Scx, Tnmd, Tnc-C and Thromb4. To recreate the level of complexity stemming from biophysical, biochemical, and biological cues of native tendon niches, hierarchical anisotropic structure directing 3D cellular orientation were produced. PCL micro and nano electrospun fibres have been evaluated to explore their role in TSCs differentiation in growth factor supplemented media.

RESULTS: We demonstrated that MSCs tenomodulin expression was improved by GDF7 up to 134% with respect to control in 21%O₂ and up to 78% in 2%O₂. In TSCs, instead, we noticed that physoxia, independently from any other exogenous stimuli, can preserve the tenocyte's phenotypic profile. 'Green' electrospun scaffolds with micron range fibres maintained tenocytes phenotype, alignment and elongation. Further, TSCs seeded on 1±0.4 µm fibres displayed increased extracellular matrix deposition.

DISCUSSION & CONCLUSIONS: Tendon tissue engineering aims to provide autograft alternatives in providing a biocompatible scaffold for cell and tissue remodelling in vivo. Implementing three dimensional scaffolds aids in guiding and controlling cell orientation, ultimately enabling anisotropic tissues in vitro. Engineering artificial platforms intended to sustain tissue production in vitro must take into account the dynamic structural and compositional changes of the system. The array of signal embossed on the material surface constitutes the initial condition from which cell-cell and cell-matrix interactions establish and guide the evolution of the entire array of signals.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Drug delivery

OP-004

Proteomic analysis of doped sol-gel coatings: correlation between *in vitro* and *in vivo* biological responses

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INTRODUCTION: Poor correlation between the results of *in vitro* testing and the subsequent *in vivo* experiments hinders the design of biomaterials. Thus, new characterisation methods are needed. This study used proteomic and histological techniques to analyse the effects of Ca-doped biomaterials *in vitro* and *in vivo* and verify the correlation between the two systems.

METHODS: The acid catalysis sol-gel route was employed to synthesise the different coatings, from the combination of MTMOS and TEOS alkoxysilane precursors. This material was functionalised with 0.5 and 5 wt% of CaCl₂. Ti discs and custom-made implants were coated with prepared materials by dip-coating. Morphology of the coatings was examined using SEM; the Ca²⁺ ion release from the materials was analysed by means of ICP-AES spectroscopy. The osteogenic and inflammatory responses were inspected *in vitro* in

human osteoblasts (HOb) and TPH-1 monocytes. The *in vivo* experiments used a rabbit model. The nLC-MS/MS-based proteomic methods were utilised to analyse the proteins adhering to the material samples incubated with human serum or examine protein expression in the tissues close to the implants.

RESULTS: Ca-doped biomaterials caused a remarkable increase in the adsorption of coagulation-related proteins, both *in vitro* (PLMN, THRB, FIBA and VTNC) and *in vivo* (FBLN1, G1U978). Enhanced affinity to these materials was also observed for proteins involved in inflammation (CO5, C4BPA, IGHM and KV302 *in vitro*; CARD6, DDOST and CD14 *in vivo*) and osteogenic functions (TETN, PEDF *in vitro*; FBN1, AHSB, MYOC *in vivo*).

DISCUSSION & CONCLUSIONS: The results obtained using different techniques were well matched, with a good correlation between the *in vitro* and *in vivo* experiments. Thus, the proteomic analysis of biological responses to biomaterials *in vitro* is a useful tool for predicting their impact *in vivo*.

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Keywords: Bone and bone disorders (osteoporosis etc), Omics / bioinformatics and systems biology

OP-005

Endotoxin Removal from Chitosan Produces Biocompatible Chitosan-Genipin Hydrogels

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INTRODUCTION: Chitosan-based biomaterials are becoming increasingly popular for biomedical applications including wound healing, drug delivery and tissue regeneration. As chitosan is naturally derived, it is susceptible to endotoxin contamination. Bacterial endotoxins elicit potent proinflammatory responses *in vivo*, therefore, it is critical that endotoxin is quantified and removed from any biomaterial intended for *in vivo* use. In the presented work, heat-treatment (180°C for 1.5 hours) and sodium hydroxide-treatment (1M for 2 hours) are investigated as two endotoxin removal methods from chitosan. While endotoxin removal is the prime task, it is important to note that preservation of chitosan structure is vital for synthesis and *in vivo* lysozyme degradation of chitosan-based hydrogels.

METHODS: The limulus amebocyte lysate (LAL) assay was used to quantify the endotoxin content of chitosan samples. Endotoxin removal was also assessed by measuring TNF-alpha production from PBMCs cultured with treated or native chitosan. FTIR was employed to determine the effect of endotoxin removal methods on chitosan structure. Chitosan-genipin hydrogels were synthesized and lysozyme degradation was investigated gravimetrically. Hydrogels were co-cultured separately with immature monocyte-derived DCs (moDCs) and cell viability and expression of maturation markers (CD83, CD86 and PDL-1) expression was assessed by flow cytometry.

RESULTS: Both heat and NaOH treatment significantly reduced the endotoxin level compared to native chitosan, to concentrations below the FDA limit for medical devices (0.5 EU/ml). NaOH-treatment significantly reduced TNF-alpha secretion by PBMCs compared to native chitosan; however, the difference was non-significant for the heat-treated chitosan, which was excluded from further testing. Although the FTIR spectra of native and NaOH-treated chitosan were extremely similar, NaOH treatment reduced the degree of acetylation of native chitosan by 6%. As lysozyme only interacts with the acetylated units of chitosan, it was important to determine if NaOH-treated chitosan is susceptible to lysozyme degradation. Chitosan-genipin hydrogels were synthesized and gravimetric degradation studies using lysozyme showed that hydrogels composed of native and NaOH-treated chitosan had comparable degradation rates. NaOH-treated chitosan did not negatively affect viability of immature DCs, furthermore the expression of maturation markers was non-significant between NaOH chitosan-genipin hydrogel condition and the immature DC control.

DISCUSSION & CONCLUSIONS: NaOH treatment is a cheap, effective endotoxin removal method that preserves chitosan structure. Resultant chitosan-genipin hydrogels synthesised with NaOH-treated chitosan are biocompatible with moDCs, as they do not induce cell death or maturation.

Keywords: Biomaterials, Hydrogels and injectable systems

OP-006

CELL GUIDING FIBROIN/CHITOSAN FILMS MADE by ATMOSPHERIC PLASMA DEPOSITION

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INTRODUCTION: Natural polymers are largely proposed as bioactive coatings but their application to surfaces are limited by several factors, such as limited control on the mechanical and chemical stability and weak adhesion to the underlying surface. [1]

Plasma process provides unique features, such as surface activation, functionalization or assisted polymerization, which can be obtained using mild conditions. Plasma modification can enhance the adhesion via covalent bonding between functional the groups forming at the interface between the substrate and the coating. On the other side, commonly adopted cold plasma processes imply limited coating thickness and topography control.

In this research, we present a new methodology to obtain spatially controlled deposition of natural biopolymers (silk fibroin and chitosan) using an atmospheric plasma torch fed by an aerosol aqueous solution containing the polymers to be deposited [2].

METHODS: The resulting coatings were characterized by electron and atomic force microscopy and ATR-FTIR. The stability of the films were tested in phosphate-buffered saline (PBS) solution (pH 7.4) for 2 weeks at 37 °C, followed by treatment in sonication bath for 10 min. Adhesion strength was evaluated by peeling test.

RESULTS: The presented plasma process provides unique features in one step, such as surface activation, functionalization or assisted polymerization. Coatings can be obtained using extreme low power (10 W) and room temperature, resulting having excellent adhesion and stability on a large variety of materials, even with complex shape geometries: a soda-lime glass, a metal alloy (Ti4Al6V), a thermoplastic polymer (polyethylene terephthalate), a silicone rubber (poly-dimethylsiloxane), without any need of surface pretreatment.

The developed method was also successfully optimized for multiple layer deposition of fibroin-on-chitosan and chitosan-on-fibroin, with the aim to realize patterned surfaces.

The biological response of these patterned surfaces were then tested for protein adsorption and cell culture experiments, evidencing their capacity to guide cell adhesion and control cell proliferation.

DISCUSSION & CONCLUSIONS: This method allows to achieve spatially controlled deposition, even on complex shaped substrates, together with deposition of different biomaterials in mild condition. This versatility can represent a powerful method for obtaining instructive biosubstrates with optimized tissue interaction, suitable for application in biomedical implants and bioelectronics devices.

ACKNOWLEDGEMENTS: This project has received funding from the Italian Ministry for Education, University, and Research (MIUR) through the "Department of Excellence" program.

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Keywords: Biomaterials, Interfaces - engineered

OP-007

Therapeutically targeting cell function using organosolv lignin – an abundant and emerging sustainable biomass source of raw material for tissue engineering strategies

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INTRODUCTION: Lignin biomass is an emerging sustainable source of raw material. It can be used as an eco-friendly precursor for innovative biomaterials and its utilization in the biomedical tissue engineering field is exponentially growing due to interest in the conversion of abundant biogenic waste with chemical value into valuable materials. We investigated whether lignin could be used to control the phenotype of dedifferentiating and diseased chondrocytes for its use in cartilage tissue engineering applications. Lignins have diverse therapeutic properties but these properties depend on the biomass source and method of extraction, which can affect the physical and chemical properties of lignin. Organosolv lignins (OL) are extracted via a green-processing approach to obtain high yields of pure lignin that resemble its native molecular structure. We recently characterized fractionated OL from hardwood and showed that the low molecular weight (MW) fraction of OL had more numerous aliphatic hydroxyl functionalities and condensed phenolic structures, a less branched conformation and an increased hydrogen bonding capacity vs. a high MW fraction and was biocompatible with several cell types commonly used in tissue engineering. Due to these characteristics, we hypothesized that low MW OL could promote intermolecular interactions with chondrocytes and thereby modify the phenotype of diseased chondrocytes by changing the cell morphology and phenotype into a healthier phenotype.

METHODS: Diseased chondrocytes were isolated from osteoarthritic (OA) cartilage tissue. Passage 1 chondrocytes from n=4-8 different donors were treated with low MW OL.

RESULTS: In already diseased OA chondrocytes, low MW OL significantly decreased the gene expression levels of COL1A2, an unhealthy phenotypic marker of OA and showed a trend in increasing COL2A1, a healthy phenotypic marker. Thus, low MW OL significantly modified multifactorial aspects of chondrocyte cell morphology and induced a less fibroblastic cell morphology and therefore healthier cell shape, which correlated with positive changes in the gene expression. Incorporation of low MW OL into hydrogels significantly increased scaffold stiffness and viscosity as well as chondrocyte attachment, demonstrating biocompatibility of low MW OL in a tissue engineering scaffold.

DISCUSSION & CONCLUSIONS: This can open up new possibilities for using OL in tissue engineering strategies for therapeutically targeting cell function.

Keywords: Cartilage / joint and arthritic conditions, Biologics and growth factors

OP-008

Extracellular Vesicles for bone repair/regeneration: modulating the cross-talk between immune cells and Mesenchymal Stem/Stromal Cells

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INTRODUCTION: Bone defects and fractures caused by injury or trauma require hospital treatment and often temporary loss of mobility, representing an important economic burden for societies and health systems worldwide. Non-union or delayed union occur in 5-10% of fractures, these are worsened by ageing and chronic inflammation, and current therapies cannot overcome this challenge. New cell-based therapies for bone regeneration are being researched at pre-clinical and clinical levels, mostly involving Mesenchymal Stem/Stromal Cell (MSC) transplantation. Yet, clinical use of MSC involves important risks, and MSC positive impact in tissue repair, including their immunomodulatory role, are ascribed to paracrine factors, particularly their secreted Extracellular Vesicles (EV)[1]. We previously showed that Dendritic Cell (DC)-EV can recruit MSC[2], but their role in inflammation to repair transition remains unknown. Herein we aimed to study the impact of bone injury microenvironment on immune cell produced EV and their cross-talk with MSC.

METHODS: Bone Marrow DC and macrophages were obtained from a rat bone defect model, 3 and 14 days after injury. Rat MSC and skin fibroblasts were isolated and characterised. EV were produced in media containing EV-depleted FBS, isolated by differential (ultra)-centrifugation, and characterised by transmission electron microscopy, nanoparticle tracking analysis and Western blot. Cell recruitment was investigated by transwell migration assays.

RESULTS: The results obtained show that the ability of DC-EV to recruit MSC changes significantly with time after bone injury. EV secreted by DC obtained 3 days after bone injury impaired MSC migration, while those from DC obtained 14 days after bone injury significantly promoted MSC recruitment. DC-EV were more efficient in recruiting MSC than macrophage-EV. Moreover DC-EV did not impact MSC proliferation or differentiation and did not promote fibroblast recruitment.

DISCUSSION & CONCLUSIONS: These results support that EV act specifically, and confirm our previous report on DC-EV ability to recruit MSC[2].

This work contributes to the development of new EV-based therapies, to accelerate the transition between inflammation and repair, thus improving bone healing in ageing and disease.

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Keywords: Bone and bone disorders (osteoporosis etc), Immunity / immunomodulation / macrophage

OP-009

Uniaxial compression promotes osteogenic response of hBM-MSCs and suppresses osteoclastogenic response of hPBMCs in co-culture within composite scaffolds

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INTRODUCTION: Bone is a highly dynamic tissue that undergoes continuous remodeling through lifetime regulated by bone forming osteoblasts and bone resorbing osteoclasts. Mechanical stimuli applied on bone tissue can shift the balance between these two cell populations [1]. In this study, the application of uniaxial compression in a 3D co-culture comprising human bone marrow mesenchymal stem cells (hBM-MSCs) and human peripheral blood mononuclear cells (hPBMCs) was evaluated [2]. The two cell populations were seeded onto 3D scaffolds produced by fused deposition modeling. The scaffolds composition was 90/5/5 %wt of poly-L-lactic acid, polycaprolactone and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) substituted with 2.5% wt strontium-nano-hydroxyapatite. **METHODS:** hBM-MSCs (7x10⁴ cells/scaffold) were seeded and cultured under static and dynamic culture conditions for 13 days, followed by the addition of hPBMCs (50x10⁴ cells/scaffold) and application of mechanical stimulation every other day for 25 min at a frequency of 1 Hz and a strain at 8% of the scaffold side (400 µm of displacement). Cell proliferation and morphology were monitored via a reduction-based cell viability assay, scanning electron microscopy (SEM) and confocal microscopy. Measurements of the alkaline phosphatase (ALP) and tartrate acid phosphatase (TRAP) activity were conducted to determine the effect of the scaffolds on the osteogenesis and osteoclastogenesis. Quantitative polymerase chain reaction was applied to quantify gene expression changes of osteogenesis-related markers (osteonectin, osteoprotegerin and osteocalcin), and osteoclastogenesis-related markers (TRAP, dendritic cell-specific transmembrane protein and nuclear factor of activated T cells 1).

RESULTS: The cell viability assessment displays excellent biocompatibility, while SEM and confocal microscopy images display well-spread cells. After 14 and 28 days, the ALP activity is higher in the dynamic co-culture compared to the static one and the corresponding hBM-MSCs mono-culture. The TRAP activity results showed a significantly lower TRAP activity in the dynamic co-culture than in the static co-culture and the hPBMCs mono-culture after 14 days. Gene expression of osteogenesis related markers was significantly higher in the dynamic co-culture than in the static and mono-cultures of hBM-MSCs.

DISCUSSION & CONCLUSIONS: The results confirm that the mechanical stimulation enhance osteogenesis and suppress osteoclastogenesis in an in vitro cell co-culture of hBM-MSCs and hPBMCs due to the communication of the two cell populations compared to the monocultures and the static co-cultures.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Bioreactors

OP-010

Bone cell regulation of osteoclastogenesis is dependent on the stage of lineage commitment and mechanical environment

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INTRODUCTION: Osteoporosis arises in part due to an increase in bone resorption mediated by osteoclasts. This results in low bone mass, microarchitectural deterioration, and increased fracture risk[1]. Osteocytes are the most abundant cells in bone, and their role in the regulation of osteoclasts via the secretion of factors such as RANKL is well known. This regulation is dependent on mechanical loading[2]. However, other cells of the osteogenic lineage are also known to be mechanoresponsive[3,4], but the extent to which they can influence osteoclastogenesis is less well understood. Therefore, the objective of this study is to investigate the impact of bone cells along the range of lineage commitment on osteoclastogenesis.

METHODS: Human marrow stromal/stem cells (hMSCs), human osteoblasts (hOBs) and MLO-Y4 osteocyte-like cells were stimulated using parallel flow chambers to provide fluid shear at 1 Hz, 1 Pa for 2 h. Osteogenic lineage cell conditioned media (CM) was collected 24h post stimulation and used to culture human blood-derived CD14⁺ monocytes (625000 cells/cm²) in 1:1 dilution, in the presence of RANK-L (50 ng/ml) and M-CSF (25 ng/ml). Cells cultured in RANKL and M-CSF supplemented growth media were used as control (Treatment). After 7 days culture, cells were stained for TRAP activity followed by DAPI staining. ImageJ was utilized to count the number of osteoclasts defining an osteoclast as a TRAP positive cell containing 3 or more nuclei.

RESULTS: TRAP and DAPI staining indicated that paracrine factors secreted by mechanically stimulated osteocytes had exerted a strong inhibition (0.01-fold vs. Treatment) on osteoclastogenesis. A similar inhibitory effect was observed but to a lesser extent in the static cultured group (0.29-fold*Treatment). Mechanical stimulation of hOBs also impaired osteoclast differentiation (0.27-fold*Treatment). Contrarily, no inhibition was observed in the static cultured group. hMSCs were shown to regulate osteoclast differentiation as well, however the static hMSCs secretome was significantly more inhibitory (0.006-fold vs. Treatment) when compared to the mechanically stimulated group (0.65-fold vs. Treatment).

DISCUSSION & CONCLUSIONS: These findings are consistent with osteocytes acting as mechanosensors and regulating osteoclastogenesis via soluble factors. In addition, hOB and hMSCs were shown to influence osteoclastogenesis but to differing degrees. In all cases, we observed significant differences between static and stimulated conditions. Taken together, these data demonstrate that bone cell regulation of osteoclastogenesis is dependent on the stage of lineage commitment and mechanical environment.

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Keywords: Bioreactors, Biomechanics / biophysical stimuli and mechanotransduction

OP-011

Non-invasive monitoring of mesenchymal stem cells osteodifferentiation by untargeted NMR exometabolomics

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INTRODUCTION: Mesenchymal stem cells (MSCs) have become the basis of numerous bioengineering studies due to their ability to differentiate into multiple cell lineages and secrete bioactive factors essential for tissue repair.¹ Because metabolic alterations seem critical for bone regeneration, metabolomics may unveil novel information on MSCs osteodifferentiation,^{2,3} allowing their behavior to be potentially guided towards improved osteogenic commitment.^{4,5} Specifically, exometabolome analysis allows for a non-invasive way to monitor differentiation, while providing important knowledge on the cell interactions with their niche.^{3,6}

METHODS: Here, untargeted nuclear magnetic resonance (NMR) metabolomics was applied to monitor exometabolome changes of human adipose-derived mesenchymal stem cells (hAMSCs) from different donors throughout 21 days of osteogenic differentiation compared to proliferation.

RESULTS: Proliferation-related metabolic adaptations revealed requirements for specific metabolites, such as glucose and glutamine for energy supply, and the secretion of most amino acids, often after day 7 of cell culture. On the other hand, osteogenesis was characterized by a less extensive amino acid secretion and a marked lactate production. Some variations were proposed as proliferation-specific (such as glucose, glutamine, citrate, threonine and 3-hydroxybutyrate) or osteogenic-specific (such as isoleucine, glutamine and lactate), independently of the nature of the donor, although confirmation of this in additional donors is required. A preliminary articulation of endo- and exometabolome results was also attempted, enabling important correlations to be established regarding energy metabolism in hAMSCs osteodifferentiation.

DISCUSSION & CONCLUSIONS: This work paves the way to exploit metabolic markers to monitor (and potentially predict) cell proliferation and osteogenic ability for different donors.

ACKNOWLEDGEMENTS: FUNDING: BetterBone (2022.04286.PTDC) & BIOIMPLANT (PTDC/BTM-ORG/28835/2017), through COMPETE2020/FEDER (POCI-01-0145-FEDER-028835); CICECO-Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020) through FCT/MCTES (PIDDAC); SFRH/BD/150655/2020; National NMR Network (Project N°022161) through FEDER (COMPETE 2020/POCI/PORL/FCT (PIDDAC)).

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Keywords: Differentiation, Omics / bioinformatics and systems biology

OP-012

Posterolateral spinal fusion in sheep induced by rhBMP6 in autologous blood coagulum with synthetic ceramics

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INTRODUCTION: Posterolateral spinal fusion (PLF) is a surgical procedure used for the treatment of degenerative spine conditions including degenerative disc disease, spinal instability, spondylolisthesis, and symptomatic scoliosis. Osteogrow-C is a novel osteoinductive device containing recombinant human Bone Morphogenetic Protein 6 (rhBMP6) delivered within autologous blood coagulum (ABC) with the addition of synthetic calcium phosphate (CaP) ceramics. The Sheep PLF model is an advanced animal model for the final preclinical evaluation of promising therapeutic solutions for achieving spinal fusion. This study aimed to evaluate the safety and efficacy of Osteogrow-C implants in the sheep PLF model.

METHODS: The experiments were conducted on 21 sheep (*Ovis aries*, Merinolaandschap breed, aged 3 to 5 years, and weighing 50–80 kg) and the experimental design was approved by the Institutional and National Ethics Committee. Osteogrow-C implants were prepared as follows: lyophilized rhBMP6 was dissolved in water and added to autologous blood withdrawn from the jugular vein. Blood was mixed with CaP ceramic particles (size: 74–420 µm or 500–1700 µm; chemical composition: TCP-80%/HA-20%) and left to coagulate. Sheep lumbar spine was exposed and following decortication of transverse processes Osteogrow-C implants were placed bilaterally between L5–L6 transverse processes. Sheep were terminated 14, 27, or 40 weeks following surgery. Spinal fusion outcome was evaluated by in vivo CT scans 9 weeks after surgery and ex vivo on microCT and histological sections as well as by biomechanical testing.

RESULTS: Osteogrow-C with both 74–420 µm and 500–1700 µm ceramic particles induced bone formation and spinal fusion after 9 weeks as observed on in vivo CT scans. Osseointegration of newly formed bone with native transverse processes was confirmed on ex vivo microCT sections. MicroCT analyses have revealed that there was no difference in the induced bone volume among tested Osteogrow-C formulations. On the other hand, the residual volume of ceramics was lower in implants with 74–420 µm than 500–1700 µm particles indicating a higher resorption rate of smaller ceramic particles. Histological analyses have shown that Osteogrow-C induced bone at the surfaces and between ceramic particles as well as cortices forming continuity with transverse processes. Three-point bending test confirmed that the induced fusion was biomechanically competent.

DISCUSSION & CONCLUSIONS: Osteogrow-C successfully promoted fusion between adjacent transverse processes in the sheep PLF model providing hope that it might be a safe and efficacious therapeutic solution in clinics for patients with degenerative diseases of the spine.

Keywords: Biomaterials, In vivo and animal models

OP-013

Biofabrication of clinically relevant osteochondral implants

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INTRODUCTION: Current surgical treatments provide sub-optimal tissue repair for articular cartilage defects. Multiple biofabrication approaches have tried to recreate the chondral biochemical and structural composition, which has frequently been combined with an osteal anchor in the form of an osteochondral plug. However, these plugs are relatively small (diameter = 6 mm) and do not recapitulate the anatomical shape of a knee joint. In this study, the translation from osteochondral plug to larger clinically-relevant and patient-specific implants is explored.

METHODS: Patient-specific, large (surface area= 469 mm²) implants consisted of a porous bone component and microfibre reinforced, cell-laden gelatin methacryloyl (gelMA) cartilage component. The bone component (a combination of magnesium and polycaprolactone (PCL)[1]) was bioprinted with extrusion-based bioprinting. Using melt electrowriting (MEW), the resurfacing cartilage component (reinforcing boxes of polycaprolactone) was made. The resurfacing printing path was automatically determined, based on an STL file of the defect. Goat-derived articular cartilage derived chondroprogenitor cells (ACPCs) were encapsulated in gelMA (density=20*10 cells/ml). Implant size (bone component only) versus stress-strain distribution was studied by axial compression and compression under 50- and 60-degrees flexion angles using a robotic arm system (Instron5967, USA). The effect of implant size on cartilage-like tissue matrix formation (glycosaminoglycans (GAGs) and collagen type II) and subsequent mechanical properties was analyzed.

RESULTS: Implant stiffness and maximum force were inversely proportional to implant size. Large implants show lower stiffness (40MPa versus 98MPa), lower maximum force (76N versus 187N), and more permanent deformation (up to 13%) as compared to small implants (0%). To completely resurface the bone-component with reinforcing MEW fibres, the printing trajectory was slightly adjusted. An increase in size of the cartilage component (diameter=6–24mm) did not hamper cartilage-like tissue formation in vitro. The relatively large, anatomically shaped implant showed a smooth surface and cartilage-like matrix distribution after in vitro culture, reflected by positive safranin-O and collagen type II staining as well as with quantitative biochemical assessment.

DISCUSSION & CONCLUSIONS: This study shows the first steps in the translation from an osteochondral plug towards larger patient-specific implants for the treatment of joint damage. The

implant size affects the stiffness (under axial loading) and maximum force (under flexion-extension) of the bone component of the implant. Yet, the successful resurfacing, matrix formation, and mechanical properties of the full construct are promising for moving towards clinically relevant, patient-specific, and mechanically stable (osteo)chondral implants.

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REFERENCES: [1]Golafshan et al.2020

Keywords: Cartilage / joint and arthritic conditions, Biofabrication

OP-014

Human Engineered and Decellularized Cartilage Grafts Steer Bone Regeneration in Critical-Sized Orthotopic Defect in Immunocompetent Models

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INTRODUCTION: We previously described the MSOD-B, a customized hMSC line over-expressing BMP-2 and capable of generating reproducible hypertrophic cartilage matrices (HyC)¹, which after decellularization (dHyC) exhibited exceptional osteoinductive properties when ectopically implanted in immunodeficient (ID) mice. While ID mice are considered standard for assessing the regenerative capacity of bone grafts, their relevance towards preclinical validation is in question due to a lack of an efficient immune system. Here, we investigate its regenerative potential and immunogenic response ectopic and orthotopically in small immunocompetent (IC) mice and rats.

METHODS: Our graft consists of in vitro-engineered cartilage tissue produced by the MSOD-B. Subsequently, the tissue is decellularized by combining detergent (SDS) and DNase washes. The osteoinductivity of decellularized grafts was assessed through subcutaneous implantation in ID and IC mice for 12 weeks. Recruitment of immune cells is investigated on day 3/7/10 post-implantation in both ID and IC mice. The regenerative potential was evaluated in a critical-sized femoral defect (5 mm) in Sprague Dawley rats. Bone repair was assessed 6- and 12 weeks post-implantation through histological, micro-computed tomography (μ CT), and mechanical analyses.

RESULTS: After 12 weeks of implantation, in vivo bone remodeling was found by μ CT and histology in the ectopic site in ID mice and the critical-sized femoral defect in an immunocompetent rat model, with full-bridge after six weeks and complete bone repair 12 weeks post-implantation with restored mechanical properties. Interestingly only a minor calcification could be found in IC mice. Correlated to the early immune response, no significant differences were observed when ID or IC was compared.

DISCUSSION & CONCLUSIONS: Our study illustrates the capacity of exploiting MSOD-B to produce osteoinductive decellularized cartilages. In addition, the strategy employed, from ectopic to orthotopic, allows us to verify their potential and brings us one step closer to a possible clinical trial. We further show the importance of a relevant animal model to evaluate the efficacy of clinically targeted products.

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<https://doi.org/10.1002/adma.202103737>

Keywords: Decellularised matrices, Bone and bone disorders (osteoporosis etc)

OP-015

Towards A Physiologically Relevant Lung In Vitro Model for Early Cancer Biomarkers

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INTRODUCTION: Although compartmentalised breathing lung in vitro models exist, the scaffolds used to approximate the native lung tissue can be improved. This study aims to fabricate a membrane with lung-specific structural and mechanical properties that facilitates lung epithelial cell culture. The model will be used to model early-stage lung adenocarcinoma in a physiologically-relevant environment including breathing motion.

METHODS: A fibrous membrane was fabricated by electrospinning thermoplastic polyurethane (Lubrizol, USA). Morphological analysis of the membrane was carried out in ImageJ. Mechanical testing was carried out on a uniaxial tensile instrument under dry conditions. Lung cell compatibility was tested by alamarBlue assay using the H441 cell line over 11 days. Formation of an epithelial monolayer was tested by immunofluorescent staining for cell-cell junction markers ZO-1 and E-cadherin. MLE 12 cells were seeded either alone or in a co-culture system comprising KRAS wild-type and KRAS G12C or G12D cells at a ratio of 3:1, respectively.

RESULTS: We have fabricated a membrane of controlled fibre diameter (range: 0.2-1 μm , $n=3$) and small pore size (1 ± 0.4 μm in diameter, $n=3$). The membrane displays good mechanical properties (Young's modulus = 11.3 ± 6.4 MPa, $n=5$) and remains elastic when subjected to human or mouse breathing simulations. Based on their metabolic activity, H441 cells can be maintained in a stable culture for 11 days ($n=3$). ZO-1 and E-cadherin markers are present on the plasma membrane in both control and polyurethane seeded cells over the 11-day period of the experiment ($n=3$). Similarly, MLE 12 cells show good attachment to the scaffold and exhibit altered morphology upon expression of the mutant KRAS.

DISCUSSION & CONCLUSIONS: Our scaffold demonstrates good structural and elastic properties, important aspects of the lung tissue and a requirement for incorporation into a breathing apparatus. We are now investigating modification of the scaffold to accommodate cyclic deformation in order to study the effect of breathing motion on the co-culture.

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Keywords: In vitro microenvironments,

OP-016

Investigating the Determinants of Breast Cancer Dormancy in Bone

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INTRODUCTION: About 70% of patients with advanced breast cancer (BC) develop bone metastasis, which usually occurs decades after the definitive treatment of the primary tumor, suggesting the presence of a cellular stage in distant sites, where disseminated tumor cells (DTCs) remain non-proliferative for a prolonged time before leading to a metastatic outbreak. This opens an exciting therapeutic opportunity to provide novel means for the prevention of metastatic disease. Despite a growing understanding of BC dormancy in bone, mechanisms that trigger the switch from dormancy to the proliferation of DTCs remain elusive. Here, we aim to unravel underlying mechanisms that play a role in controlling BC dormancy in bone.

METHODS: We engineered BC cell lines to co-express luciferase-tdTomato and a mutant reporter of p27 (mVenus-p27K-) that identifies quiescent cells. To investigate the interrelation between dormant DTCs and surrounding cellular elements, we developed in vivo and in vitro models.

RESULTS: In vivo, the co-existence of a distinct stage of metastasis was detected with IF imaging in the femur of mice after the injection of labelled 4TO7 cells. In vitro, MCF-7 cells were detected in the vicinity of CD31+ vasculature-like cells in the engineered vascularized osteoblastic niche in the perfusion bioreactor.

DISCUSSION & CONCLUSIONS: Next, we will increase the complexity of the bioengineered niche by introducing other cellular components that will be identified with the recently developed in vivo BC bone dormancy model.

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Keywords: Cancer, Bone and bone disorders (osteoporosis etc)

OP-017

Analyse The Effects Of Glycosaminoglycans On Prostate Cancer Cells Using Collagen-based Scaffolds

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INTRODUCTION: Prostate cancer (PC) is one of the leading causes of cancer-related death in men worldwide. In the early stages of advanced PC, cancer cells undergo epithelial-to-mesenchymal transition (EMT), a process whereby epithelial cells lose their cell-cell adhesion, gain migratory and invasive characteristics, and travel through the blood vessels to invade new tissue, leading to metastasis. The extracellular matrix (ECM) of prostate tissue contains various constituents, including chondroitin sulfate (CS) and hyaluronic acid (HyA), which are significantly increased in metastatic PC and are of interest for further investigation. As a result, this project aims to develop three-dimensional (3D) porous collagen/CS (Col/CS) and collagen/HyA (Col/HyA) scaffolds suitable for studying in vitro PC EMT and can be used as a platform to test and develop treatments for PC, while also reducing the need for animal models.

METHODS: Three Col/CS and two Col/HyA scaffold compositions were prepared based on previous lab protocols (Fitzgerald, et al. 2015). These scaffolds were subjected to rigorous characterisation, including mechanical testing, SEM, pore size analysis, and porosity measurements. To determine the impact of CS and HyA on PC EMT processes and metastasis growth, osteoblastic-like phenotype LNCaP and osteoclastic-like phenotype PC-3 PC cells were cultured on all scaffolds. Furthermore, biochemical evaluations, such as metabolic activity, DNA quantitation, Live/Dead cell viability assays, and histology analysis were used to determine their suitability for mimicking the ECM for PC and their ability to support cell growth and proliferation. Additionally, prostate-specific antigen (PSA) concentrations, EMT-related cytokines expression, and EMT-related gene expression were examined on the Col/CS and Col/HyA scaffolds to determine the impact of increasing CS and HyA concentrations on the EMT process.

RESULTS: Overall, highly porous Col/CS and Col/HyA scaffolds were successfully developed. Mechanical testing showed that the wall stiffness of Col/CS medium, Col/CS high, and Col/HyA high simulate the wall stiffness of PC tumours. Initial findings demonstrated that the Col/CS and Col/HyA scaffolds supported PC cell growth and proliferation, and the PC cell lines maintained their phenotype.

DISCUSSION & CONCLUSIONS: In conclusion, these scaffolds demonstrate an ideal in vitro platform for studying PC, including the associated EMT process and have great potential to act as a testing bed for the assessment of new drug treatments.

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REFERENCES: Fitzgerald, et al. Biomaterials. 2015,66:53-66.

Keywords: Biomaterials, In vitro microenvironments

OP-018

Bioengineering approaches to recapitulate the alternative vasculature in neuroblastoma

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INTRODUCTION: Neuroblastoma (NB) is a highly vascularized pediatric tumor characterized by NB cells presenting three different tumorigenic potentials: N (highly malignant), S (nonmalignant), and I-type (intermediate). NB vessels can be formed through physiological-based mechanisms such as angiogenesis and vasculogenesis. However, also, NB tumors apply an alternative strategy known as endothelial transdifferentiation, by which cancer cells acquire endothelial properties and become tumor-derived endothelial cells (TECs). TECs are critical for tumor development and metastasis. However, current antiangiogenic strategies are only designed to target physiological-based mechanisms, not alternative vasculature. This is mainly because a lack of experimental models has hampered TEC studies. Thus, it is critical to recapitulate in vitro TECs subpopulations to design a specific therapy further.

METHODS: *Polydimethylsiloxane (PDMS)-based platform.*- For mimicking the stiffness for physiological angiogenesis, Sylgard 184 reagents and Sylgard 527 reagents were mixed to get equilibrium modulus values in the range of arteries and venous tissue, respectively.

Neuroblastoma-on-a-chip set-up.- “butterfly”-like microfluidic chips were fabricated, and LUC-1 Luer and male Luer integral 1/16 connectors were used to connect Tygon tubes to chip inlets and outlets. The culture medium was pumped using a high-precision IPC-N 8 peristaltic pump.

Hydrogels preparation and characterization.- hydrogels were fabricated using bovine collagen I, fibrinogen from bovine blood plasma, and cross-linked with thrombin.

RESULTS: We mimicked the stiffness for physiological angiogenesis in vitro by developing a biocompatible PDMS-based platform of different stiffnesses. We demonstrated that the most aggressive NB cell phenotypes (N- and I-types) were mainly transdifferentiated in stiff substrates in the arteries range. In contrast, the less aggressive cell lines (S-type) lacked transdifferentiation capacity. We also recapitulated the NB physiological stiffness by fabricating a collagen I/fibrin-based hydrogel. NB cells cultured within the hydrogel, but static, did not result in transdifferentiation. However, the inclusion of shear stress into a microfluidic chip device (NB-on-a-chip model) induced the conversion of NB cells into TECs.

DISCUSSION & CONCLUSIONS: We developed two alternatives to obtain TECs in vitro. The stiffness-based PDMS platform is a simple method to predict NB aggressiveness and metastatic potential based on the capacity to transdifferentiate into TECs. On the other side, the NB-on-a-chip, with a high level of mimicry, is a complex model that makes performing high-throughput screenings of drug libraries challenging. We propose the stiffness-based platform as a previous drug-testing step before the NB-on-a-chip model to discard ineffective treatments in a way compatible with standard pharmaceutical industry equipment and protocols.

Keywords: Cancer, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-019

Liver-Specific Ligand-Conjugated Microparticles For Targeted Hepatocyte Transplantation

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INTRODUCTION: Hepatocyte engraftment is a critical challenge for liver cell therapies as the majority of transplanted cells are cleared from liver sinusoids by proinflammatory cytokines, chemokines or receptors following instant blood-mediated inflammatory reaction or after the activation of residence macrophages. Systemic injections of IL-10, an immunoregulatory cytokine, or etanercept, a TNF blocker which specifically binds to TNF- α , has potential to improve cell transplantation. Encapsulation of these molecules within microparticles (MPs) provides controlled release strategies to increase cell engraftment. However, MPs typically fabricated with commercially available polymers are non-specific to the liver, resulting in inefficient delivery of encapsulated molecules. The asialoglycoprotein receptor (ASGPR) exhibits high affinity as a galactose receptor and is the only liver-specific receptor identified on hepatocytes. We demonstrate targeted drug delivery to the liver via microparticles fabricated with galactose ligands in various approaches to achieve targeted long- and short-term release profile, suitable to promote hepatocyte survival.

METHODS: Galactosylation was achieved as either bound to the microparticle surface (surface-bound-Gal) or covalently bound to poly(lactic-co-glycolic acid) (Gal-PLGA). In vitro functional release was measured using an ELISA assay. Immunomodulatory response of MPs was performed using THP-1 differentiated macrophages. In vitro MP-cell attachment was performed using HepG2. In vivo biodistribution and dose response was performed using wild type and AhCreMdm2fl/fl mice respectively.

RESULTS: MPs fabricated from both Gal-PLGA and surface-bound-Gal provided an enhanced MP retention (>85%, compared to 40% with conventional PLGA) in vitro. Biodistribution studies have shown superior MP retention in the liver (>70%) for both MP formulations compared to commercial PLGA MPs (<30%). Gal-PLGA MPs exhibited a mean controlled daily release for up to 7 days, which is a suitable formulation for etanercept release profile in vitro. Surface-bound-Gal MPs exhibited a low initial burst release (<30%) followed by controlled daily release for up to 42 days, which is a suitable formulation for IL-10 release. In vitro functional etanercept release showed binding with TNF- α and reduces inflammation observed by down-regulated pro-inflammatory genes (CXCL2, IL-1 β) and reduced release of soluble inflammatory cytokines (TNF- α , IL-6).

DISCUSSION & CONCLUSIONS: We have developed novel synthesis routes for galactosylation of polymers and subsequently fabrication of Gal-PLGA and surface-bound-Gal MPs which were preferentially retained in the liver in vivo. MPs had controlled functional release kinetics of etanercept and IL-10 and in vitro reduced inflammation. Preliminary in vivo study has shown reduced inflammatory cues and no adverse safety concerns.

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Keywords: Drug delivery, Biomaterials

OP-020

Tissue repair with multimaterial biomedical devices fabricated from sustainable biopolymers

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INTRODUCTION: In order to produce successful biomedical devices targeted at tissue repair and regeneration it is important produce structures that attempt to mimic the missing or damaged tissue properties. This means fabricating structures resembling both the material properties, physical structures as well as incorporating live healthy cells. For this 3D printing is a popular tool in fabricating 3D structures. In this context, biopolymers i.e. Polyhydroxyalkanoates (PHAs) and alginate have exhibited exceptional value.¹⁻³ PHAs are natural biopolymers that are sustainably produced via bacterial fermentation and have been steadily gaining popularity for cell culture and tissue engineering applications due to their excellent biocompatibility.²⁻³

METHODS: PHAs were produced via fed batch bacterial fermentation and after harvesting the biopolymers were extracted via Soxhlet extraction. Extracted polymers were characterised via mechanical, NMR, FTIR, GCMS and DSC characterisation techniques to ensure there was no batch variation. PHAs were then either used in pure form for 3D Printing together with Alginate encapsulated cells or composites with Graphene nanoplatelets (GnP) were produced and printed with a Cellink Bio X Printer and seeded with cells. Produced 3D scaffolds were then cultured in vitro and further characterised with AFM and SEM.

RESULTS: We have demonstrated the ability to produce a variety of PHAs via bacterial fermentation, extracted and purified to cell culture grade. In vitro experiments with a variety of cell-lines demonstrated excellent biocompatibility. 3D Printing of PHAs together with alginate and live cells resulted in the successful fabrication of high fidelity flexible multi-material cardiac patches. Novel GnP/PHAs composites were produced and printed with high fidelity and reproducibility demonstrating excellent biocompatibility. Mechanical properties of the structures on the macro and nanoscale via AFM PeakForce microscopy on live cells are presented.

DISCUSSION & CONCLUSIONS: This work demonstrates the production of PHAs along with novel multimaterial 3D Printing together with alginate, for the production of flexible cardiac patches, to be tested in vivo and demonstrate the cell compatibility of our produced structures.³ In addition to producing a cardiac heart patch loaded with Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes (iPSC-CMs). We also successfully developed a variety of PHA and GnP composite structures via 3D printing for bone tissue engineering.

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Keywords: Polymers - natural / synthetic / responsive, Biomaterials

OP-021

Rapid Formation of Endothelial Cell Nerve Repair Constructs for Nerve Tissue Engineering

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INTRODUCTION: Traumatic peripheral nerve injury has a sudden, debilitating effect on 1 in 1000 people, resulting in loss of sensation and movement and potential long-term neuropathic pain. The current clinical 'gold standard' for peripheral nerve repair, the autograft, has multiple limitations, including donor tissue availability and donor site morbidity. Tissue engineering offers a promising alternative, artificial nerve repair constructs constituted of aligned cellular biomaterials. Whilst Schwann cells are the traditional therapeutic cell type for nerve repair, constructs containing endothelial cells have been shown to further improve regeneration. Here, the incorporation of endothelial cells into nerve repair constructs was investigated alongside the advancement of a technique for rapid production of the constructs to aid clinical translation.

METHODS: Collagen hydrogels, seeded with endothelial cells, were stabilised by controlled aspiration under negative pressure using the Gel Aspiration-Ejection (GAE) technique. Experimental setup, hydrogel casting mould, and rate of aspiration were explored to improve endothelial cell viability and alignment, and construct length and production feasibility. Viability and alignment were assessed by cell staining and lactate dehydrogenase assays, and confocal imaging of cytoskeletal staining.

RESULTS: Reducing the aspiration rate of the GAE technique when stabilising cellular collagen hydrogels, improved endothelial cell viability within constructs by 40% and significantly improved cell alignment ($p < 0.001$).

Moving to a cuboidal hydrogel mould with an aspiration angle of $\sim 45^\circ$, compared to a cylindrical mould with a $\sim 0^\circ$ aspiration angle, resulted in the ability to stabilise a larger hydrogel volume, increasing the construct length by 50%.

Replacing the manual aspirator component of the experimental setup with a syringe pump, progressed the technique towards the automated production of nerve repair constructs.

DISCUSSION & CONCLUSIONS: An innovative hydrogel stabilisation technique was successfully developed to produce endothelial cell nerve repair constructs with viability and alignment comparable to that seen in previously published Schwann cell constructs which supported nerve regeneration. The technique was also advanced towards clinical translation by the semi-automated production of constructs in a scalable manner.

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Keywords: Nervous system (brain-central-peripheral / disorders), Advanced therapy medicinal products

OP-022

Characterising notochordal and progenitor cell sub-populations within the human nucleus pulposus

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INTRODUCTION: The nucleus pulposus (NP) of the developing human intervertebral disc (IVD) is occupied by large, vacuolated notochordal cells (NCs) which are lost with maturity. Loss of NCs typically coincides with the IVD degeneration onset and associated back pain, therefore these cells are hypothesised to be important in NP homeostasis. We [1], and others [2], have previously demonstrated that the adult human NP retains NC-like/progenitor cell subpopulations. Here, we applied a marker panel to identify NC-like (CD24) and NP progenitor cell (TIE2&GD2) populations and then isolated cells (FACS) to assess phenotype. Moreover, function of CD24 was assessed using CRISPR KO technology and IL-1 β stimulation.

METHODS: Flow cytometry was used to analyse CD24, Tie and GD2 expression in a cohort of adult human NP samples across a range of ages (20-76) and degeneration grades (5-12, n=10). FACS sorting for CD24&GD2 (n=10) was then used to isolate four populations: GD2+/CD24-, GD2-/CD24-, GD2+/CD24+ and GD2-/CD24+ and cell phenotype was assessed (qPCR) for known healthy NP and catabolic markers. Lastly, CRISPR knockout (KO) technology was used to generate CD24 KO cell lines from a CD24^{high} immortal human NP cell line and functional assays were performed.

RESULTS: Flow cytometry data showed varied CD24, high GD2 and low Tie2 expression in a cohort of adult NP samples. When looking at CD24&GD2 together, a distribution of populations expressing none, either or both markers was observed. Gene expression levels of unsorted compared to the four sorted populations showed a significantly higher expression (*p<0.05) of healthy anabolic NP markers such as CD24, Col2, Brachyury and a significantly lower expression of catabolic markers (IL-1b, TNF-a and the MMPs) in the CD24+ cells. Presence of GD2 did not appear to influence gene expression. Significantly lower expression of healthy NP markers was observed in the CD24 KO cells compared to WT. Moreover, CD24 KO cells showed lower expression of anabolic and higher expression of catabolic markers following IL-1 β stimulation.

DISCUSSION & CONCLUSIONS: Presence of cells expressing CD24&GD2 suggests persistence of notochordal/progenitor cells in the adult NP. The anabolic phenotype of CD24+ sorted cells and the loss of described phenotype when CD24 is absent (KO) suggests that CD24 presence is important in maintaining a healthy notochordal phenotype. The switch to catabolism in IL- β stimulated CD24 KO cells suggests a potential role for CD24 in regulating receptor signalling through IL-1 β .

ACKNOWLEDGEMENTS: Funding body, BBSRC.

REFERENCES: [1]Sakai, D., et al.,2012; [2]Richardson, S.M., et al., 2017; [3]van den Akker, G.G.H., et al., 2014

Keywords: Intervertebral disc / spine and their disorders,

OP-023

Direct contact-mediated non-viral gene therapy using thermo-sensitive hydrogel-coated dressings

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INTRODUCTION: Nanotechnologies are being increasingly applied as systems for peptide and nucleic acid macromolecule drug delivery. However systemic targeting of these, or efficient topical and localized delivery remains an issue. A controlled release system that can be patterned and locally administered such as topically to accessible tissue (skin, eye, intestine) would therefore be transformative in realizing the potential of such strategies. We previously developed a technology termed GAG-binding enhanced transduction (GET) to efficiently deliver a variety of cargoes intracellularly, using GAG-binding peptides to mediate cell targeting, and cell penetrating peptides (CPPs) to promote uptake.

METHODS: Herein we demonstrate that GET transfection system can be used with the moisturizing thermo-reversible hydrogel Pluronic-F127 (PF127) and methyl cellulose (MC) to mediate site specific and effective intracellular transduction and gene delivery through GET nanoparticles (NPs). We investigated hydrogel formulation and the temperature dependence of delivery, optimizing the delivery system.

RESULTS: GET-NPs retain their activity to enhance gene transfer within our formulations, with uptake transferred to cells in direct contact with the therapy-laden hydrogel. By using Azowipe™ material in a bandage approach, we were able to show for the first-time localized gene transfer in vitro on cell monolayers, and also demonstrate this ex vivo in transdermal gene therapy.

DISCUSSION & CONCLUSIONS: The ability to simply control localization of gene delivery on millimetre scales using contact-mediated transfer from moisture-providing thermo-reversible hydrogels will facilitate new drug delivery methods. Importantly our technology to site-specifically deliver the activity of novel nanotechnologies and gene therapeutics could be transformative for future regenerative medicine

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Keywords: Gene therapy, Skin

OP-024

The effect of macrophage-mediated phagocytosis of nanoclay particles on macrophage phenotype and inflammatory function

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INTRODUCTION: Macrophages are crucial regulators of the immune response to damaged tissue/foreign bodies, such as biomaterials, and their initial process is an uptake of heterogeneous target particles, called phagocytosis. It is well known that particle size and shape affect macrophage phenotype and function in tissue regeneration [1]. However, the correlation between phagocytosis and macrophage phenotype/function, and their possible pathways is unknown. Nanoclay particles have recently attracted significant attention in the field of regenerative medicine as self-assembling matrices and drug-delivery modifiers [2]. In this study we investigated the effect of macrophage nanoclay particle phagocytosis on macrophage phenotype and function.

METHODS: Macrophages were isolated from mouse bone marrow (BALB/c male, 4-8 weeks) and treated with phagocytosis inhibitors (chloroquine (CQ) and cytochalasin D (CD)) at various concentrations (CQ: 100, 200, 300, 400, 500 μ M, CD: 1, 5 μ M) for 2 hours, prior to applying nanoclay particles (100 μ g/ml). Macrophage phagocytosis behaviour was observed using a time-lapse microscope and transmission electron microscopy/energy-dispersive X-ray spectroscopy. Macrophage phenotype was evaluated by flow cytometry and western blot analysis of the iNOS and CD206 expressions.

RESULTS: Nanoclay particles were observed both extracellularly and in the macrophage intracellular compartments indicating active phagocytosis. Furthermore, in the presence of 100 μ g/ml nanoclay, the M2-like macrophage population significantly increased ($p < 0.034$) compared to the control group (without nanoclay). In contrast, when treated with CD, M2 macrophages in the nanoclay-treated groups were significantly reduced compared with the nanoclay-free control – an effect not-observed with CD treatment in the absence of nanoclay. Critically, CD-treated macrophages did not actively phagocytose nanoclay particles. When actin polymerisation-related PI3K and MAPK protein expression levels were measured, PI3K expression was significantly reduced in macrophages treated with CD and cultured with nanoclay, compared to MAPK.

DISCUSSION & CONCLUSIONS: The current studies demonstrate that nanoclay-induced M2 polarisation is attenuated when macrophage phagocytosis is inhibited. The PI3K pathway is highly associated with macrophage phagocytosis-phenotype. These findings indicate that phagocytosis of biomaterials play an important role in determining macrophage phenotype/function in modulating inflammation response.

ACKNOWLEDGEMENTS: Supported by an MRC-AMED UK-Japan Regenerative Medicine and Stem Cell Research Initiative (ref.MR/V00543X/1).

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2. Kim Y-H. et al. Nat Commun. 11(1), 1-9 (2020).

Keywords: Immunity / immunomodulation / macrophage, Biomaterials

OP-025

The characterisation and antimicrobial activity of Ag-nanoparticle-incorporated eggshell membrane for dermal applications

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INTRODUCTION: Current treatments to repair and/or replace skin are severely limited. The egg shell membrane (ESM) is a unique natural material with innate physical and mechanical properties that provides optimal barrier properties and also exhibits biocompatibility/inherent biodegradability. [1, 2]. The ESM composes of three distinct layers: the inner and the outer membranes (fibrous structures) and the limiting membrane (dense structures). The ability to separate the ESM from the eggshell is vital to its application and various methods of extraction have been employed which led to differences in their final composition, structure, biological characteristics and wound healing effects [2]. In this study, ESMs were extracted using different techniques and further enhanced by incorporating with either commercially available silver nanoparticles (AgNP) [Sigma-Aldrich] or, novel AgNP manufactured by means of a unique green patented process [Metalchemy]. These modified samples were thereafter profiled in terms of the physical, biological and antimicrobial characteristics with an ultimate view for dermal applications.

METHODS: ESM samples were prepared using either a manual and/ or an optimised acid decellularization protocol. The ESMs were incubated with AgNPs solution for 20 hours and air-dried at room temperature (~19°C). The deposition of the AgNP on the ESM was confirmed using FT-IR and SEM. Metabolic activity assessment (i.e., MTS assay), LDH-release profiles and Live/Dead staining demonstrated good attachment and spreading to the samples, and high cell viability following 5 days of culture. The chicken embryo chorioallantoic membrane (CAM) assay was also employed to ascertain the angiogenic potential of the AgNP-ESMs. Assessment of the antimicrobial activity of the enhanced AgNP-ESMs were validated using the International Standard ISO 16869:2008 methodology and exploited *Cladosporium* as the test microorganism ($\geq 5 \times 10^6$ cells/ml).

RESULTS: AgNP-ESMs samples were successfully fabricated and fully characterized. HDFs (PCS-201-012) and BJ (CRL-2522) cells cultured on the AgNP-ESMs samples demonstrated high biocompatibility in terms of high cell attachment, spreading, viability and proliferation rates/characteristics and also allowed the promotion of new blood vessels (i.e., pro-angiogenic) in the CAM assay. The AgNP-ESMs exhibited good antimicrobial activity with the Metalchemy-modified samples demonstrating an exceptionally strong inhibitory effect.

DISCUSSION & CONCLUSIONS: In summary, AgNPs were successfully loaded into the ESMs extracted with different methods. The AgNP-ESMs developed exhibited desirable physical and biological properties for clinical/therapeutic dermal wound dressing and, in context of large-scale commercialization, aspects of “green process technology” and sustainability makes this an exciting opportunity.

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[2] Mensah et al, Biomaterials, 2021, 36 (2), 912-929

Keywords: Polymers - natural / synthetic / responsive, Wound healing

OP-026

Induced pluripotent stem cell-derived hematopoietic stem cell niches as standardized 3D model for human hematopoiesis

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INTRODUCTION: Human hematopoietic stem cell and progenitor cell (HSPC) niches are provided by the bone marrow (BM) tissue, but they remain poorly understood. Our lab has engineered different 3D in vitro models of human BM niches with patient-derived cells¹⁻²⁻³. However, primary cells are subjected to donor variability and are not suitable to model pathophysiological hematopoiesis in highly reproducible settings. Our goal is to engineer 3D standardized HSPC niches by replacing primary cells by human induced pluripotent stem cells (hiPSCs).

METHODS: We cultured hiPSCs to form aggregates and induced a mesoderm differentiation. These aggregates were then either cultured in 2D for 12 days or seeded on 3D hydroxyapatite scaffolds and cultured for 4 weeks to induce osteogenic and vasculogenic differentiation. hiPSC-derived CD34+ HSPCs⁴ were then cultured for 1 week in control niches engineered with primary BM mesenchymal stromal cells or in hiPSC-derived niches. These engineered niches were finally analyzed at gene (qPCR), cellular (FACS) and tisular level (histology).

RESULTS: Our 2D data reveal that hiPSCs can give rise simultaneously to CD31+ endothelial cells, CD146+ perivascular cells and CD31-CD146- osteogenically-differentiated mesenchymal cells using a single medium composition with osteogenic and angiogenic factors. In 3D, hiPSC aggregates colonize uniformly the scaffold and secrete an abundant osteogenic extracellular matrix embedding hiPSC-derived niche cells. A subset of hiPSC-derived HSPCs is maintained in 3D niches, but most of them differentiate into myeloid and megakaryocytic-erythroid lineages, recapitulating native bone marrow cellular diversity.

DISCUSSION & CONCLUSIONS: hiPSCs can be used as cell source to engineer 3D standardized human hematopoietic niches including osteogenic, endothelial, perivascular and hematopoietic cells.

Following a modular approach, we envision the possibility of engineering multiple niche configurations by adding hiPSCs differentiated towards different lineages at specific time points.

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Keywords: Induced pluripotent stem cells, Stem cell niche

OP-027

Mimicking the human vascular stem cell niche - development of different *in vitro* models

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INTRODUCTION: Hematopoietic stem and progenitor cells (HSPCs) constantly supply the human body with new blood cells. *In vivo*, HSPCs are located in highly specialized 3D microenvironments, their so-called niches. Commonly, two HSPC niches are described: the endosteal and the vascular niche. This study aims to develop *in vitro* models of the latter, using human HSPCs, isolated from the umbilical cord, together with mesenchymal stem/stromal cells (MSCs) and endothelial cells (ECs), as they are the most essential supporter cells for HSPCs in the vascular niche *in vivo*.

METHODS: In one approach, a fibrinogen scaffold is used to mimic the basal lamina. The fibrous biomaterial is seeded with cells from both sides and the HSPCs can then be introduced to either the MSC or the EC side to imitate residing in or homing to the niche. A second approach is based on cell sheet engineering (CSE). The HSPC supporting cells are cultured on thermoresponsive surfaces, which allow a temperature-triggered cell detachment, keeping them within their extracellular matrix. During detachment, both cell sheets are sequentially rolled on a gelatin template to obtain a tubular 3D-structure. Afterwards, the HSPCs can be injected into the lumen. Additionally, co-cultures of HSPCs and induced pluripotent stem cell derived ECs (iPSC-ECs), which are generated from cord blood as a juvenescent cell source, are performed. Incorporation of these early ECs into the developed models could provide insights on the proceedings in fetal vascular niches. After several days of co-/tri-culture, the cells are subjected to confocal laser-scanning microscopy (CLSM). Furthermore, medium supernatants are analyzed regarding secreted cytokines. To investigate HSPC proliferation and differentiation, flow cytometry experiments and colony-forming unit assays are conducted.

RESULTS: Both vascular niche models were established and optimized to suit the requirements of the HSPC environment. The two-sided seeding of the fibrinogen scaffold was accomplished and confirmed by CLSM. Using CSE, 3D vessel-like structures containing an inner layer of ECs surrounded by a MSC-layer were fabricated and validated using CLSM. These models enable the investigation of the influence of a 3D environment on HSPCs and the supportive effect of MSCs and ECs. With the iPSC-EC co-culture experiments valuable knowledge on cellular interaction in potential *in vitro* fetal niches was gained.

DISCUSSION & CONCLUSIONS: Utilizing the established models, it is possible to shed light on the interaction of different cell types in the vascular niche. An artificial HSPC niche could also serve as valuable reproducible platform for drug testing, being easily accessible for analysis.

Keywords: Microenvironment and niche engineering, In vitro microenvironments

OP-028

Reconstruction of the erythroblastic microenvironment in a macroporous 3D hydrogel based bone marrow analog

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INTRODUCTION: The possibility to generate erythrocytes *in vitro* has received much interest in research within the last years. Mimicking the erythroblastic microenvironment by providing artificial niches for the *in vitro* generation of mature erythrocytes from hematopoietic stem cells is a promising strategy to gain new insights in the biology of hematopoietic differentiation and has the potential to make a major contribution towards the availability of safe blood products.

METHODS: To reflect physiological conditions of the erythroblastic niche, donor-matched CD34⁺ human umbilical cord blood derived erythroblasts and CD14⁺ macrophages were seeded into macroporous 3D poly (ethylene glycol) diacrylate hydrogel scaffolds for 12 days and maturing erythroblasts were analysed via flow cytometry for the expression of the transferrin receptor (CD71), the late erythrocyte marker glycophorin A (CD235a) and their enucleation capacity. Moreover, immunofluorescence staining and the analysis of released growth factors and cytokines allowed a deeper insight in island structures and cell-cell interactions occurring within a 3-dimensional environment.

RESULTS: After a total cultivation period of 21 days, we could confirm that both, the conventional 2D and the 3D co-culture, enhanced erythroid differentiation compared to single culture conditions. The developed 3D microenvironment promoted rapid erythroid maturation, however, the presence of macrophages diminished this effect over time. We observed a decrease of the pro-inflammatory cytokines TNF- α , IL-10 and CCL1 in 3D single and co-cultures, which further emphasised the beneficial effect of the developed scaffold on erythroid differentiation. Interestingly, single cultures of erythroid precursors were either non-viable or developed a self-sustaining adherent CD14⁺/CD86⁺ population with macrophage-like cell morphology that sustained erythroid survival.

DISCUSSION & CONCLUSIONS: The introduced 3D culturing system provides an optimized biomimetic environment for the occurring cell populations that allows autocrine/paracrine signalling between erythrocytes and macrophages. In this way, we intend to get one step closer to the goal of an artificial erythroblastic niche for red blood cell generation.

ACKNOWLEDGEMENTS: This work has been carried out within the framework of the SMART BIOTECs alliance between the Technical University Braunschweig and the Leibniz University Hannover. This initiative is supported by the Ministry of Science and Culture (MWK) of Lower Saxony, Germany and funded by the Lower Saxony Ministry of Science and Culture (Niedersächsisches Vorab, VW-ZN3440) for the REBIRTH-Centre. The authors would like to thank the Soft Matter Synthesis Laboratory at the Karlsruhe Institute of Technology for preparing the polymer.

Keywords: Stem cell niche, Biomaterials

OP-029

Engineering a standardized human hematopoietic niche using a dedicated mesenchymal line and a 3D-printed bioreactor system

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INTRODUCTION: Hematopoietic stem and progenitor cells (HSPCs) constitute the mainstay of blood cells' life-long production. The diversification of HSPCs progeny is orchestrated via a process called hematopoiesis. Its regulation is achieved by complex cellular, molecular, and physical cues defining the hematopoietic niche. However, how these niche elements interact to modulate human HSPCs fate remains largely elusive. Existing in vitro approaches rely on complex systems and primary materials, leading to reproducibility issues in hematopoietic modeling. To this end, we here propose the development and exploitation of a standardized platform aiming at recapitulating the human bone marrow niche ex vivo.

METHODS: Our strategy relies on a versatile 3D-printed perfusion bioreactor for the generation of custom hematopoietic niches.

RESULTS: When combined with our human mesenchymal cell (MSC) line, we could demonstrate homogenous colonization of 3D collagen scaffolds and establishment of niches supporting HSPC populations maintenance. The system enables the recapitulation of the interactions between human HSPCs, human MSCs and their extracellular matrix in a relevant and modular 3D setting. We further exploited our system to decipher the modality and the functional impact of MSCs-to-HSPCs mitochondrial transfer. We observed a preferential transfer to hematopoietic stem population with an higher rate of transfer per HSPC in 3D.

DISCUSSION & CONCLUSIONS: Taken together, we report the validation of essential building components for the standardized but versatile generation of human 3D bone marrow proxy. The system can be exploited towards interrogating the impact of niche-presented hematopoietic cytokines or organelle transfer on HSPCs function. Our data confirm that perfusion bioreactors fit for cell culture can be generated using a 3D printing technology and exploited for the 3D modeling of complex stem cell systems. Our approach opens the gates for a more faithful investigation of cellular processes in relation to a dynamic 3D microenvironment.

Keywords: Bioreactors, Stem cell niche

OP-030

Breast-to-bone in vitro model: effect of breast tissue stiffness on invasion

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INTRODUCTION: Advanced stages of breast cancer progression have a high chance to develop incurable metastasis to bone. Upon tumour initiation, a dysregulation of the normal physiology occurs at the primary tumour site. In breast cancer, tumour cells at the primary site experience mechanical stresses in the form of an increased tissue stiffness and dense tissue architecture; as consequence, enhanced breast cancer cells migration affects the invasion to secondary sites. To better understand the link between breast and bone tissues, and how mechanically conditioned breast cancer cells invade the bone tissue and form metastases, new in vitro models are required. In this study, we have assembled two distinctive 3D scaffolds and modelled the breast-bone axis: an alginate-based hydrogel encapsulating human breast cells was used to model the tumour breast microenvironment; and polycaprolactone (PCL)-based 3D-printed scaffolds pre-conditioned with human osteoblast to model the normal bone tissue [1].

METHODS: We designed a small library of alginate-based hydrogels, varying their compressive moduli and the protein content to mimic the properties of the breast tumour microenvironment. Alginate-based hydrogels were used to better understand cause-effect relationship of tissue stiffness on metastatic potential of breast cancer cells. MDA-MB231 breast tumour cells were used to study several aspects of metastatic cascade, including cell adhesion, migration, 3D invasion and secondary site colonization, as function of the hydrogel used. In parallel, we screened different 3D PCL-composite scaffolds (including bioceramics as particles), printed to mimic bone tissue characteristics. SaOs-2 cells activity was used to select the optimal composite scaffold: evaluating the deposition of bone extracellular matrix (ECM) and calcification. Selected bone-mimicking scaffolds were assembled with hydrogels breast scaffolds to create a new biohybrid 3D scaffolds to study the breast-2-bone metastasis axis.

RESULTS: Both migration and 3D collagen invasion increased when MDA-MB231 cells were cultured in hydrogels with higher stiffness. Additionally, PTHrP expression and IL-6 release, which are implicated in bone metastatic cascade, were observed to be higher when cells from higher stiffness hydrogels were cultured in bone ECM enriched PCL scaffolds.

DISCUSSION & CONCLUSIONS: These results suggest that new models, integrating multiple tissues and their properties, are required to advance knowledge on cancer progression, enabling further insights on metastasis pathways and towards new therapeutic targets.

REFERENCES: [1] DOI: 10.1002/adhm.202201898

Keywords: Cancer, In vitro microenvironments

OP-031

Expansion culture of bone marrow-derived mesenchymal stem cells in the micro-scaffold Nichoid to treat pediatric lung disease

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INTRODUCTION: Mesenchymal Stem Cells (MSCs) represent a promising source for stem cell therapies, in particular in the field of pediatric respiratory system diseases [1]. Characterized by low immunogenicity, high anti-inflammatory and immunoregulatory features, these cells demonstrated excellent therapeutic profile in numerous in vitro and preclinical models [2]. Indeed, MSCs reside in a specialized physiologic microenvironment, characterized by a unique combination of biophysical and biochemical cues. The 3D micro-scaffold Nichoid stimulates the autocrine production of the stemness cues present in the native MSC niche, allowing to reduce the use of biochemical and xenogenic growth factors and to maintain stem cell potential by avoiding spontaneous cell differentiation [3].

METHODS: We expanded human pediatric bone marrow MSCs (BM-MSCs) inside the Nichoid and performed a complete cell characterization with different approaches as metabolic assays, immunofluorescence analysis, Western Blot and a complete RNA-sequencing with further bioinformatic analysis. Moreover, we analyzed by mass spectrometry the secretome produced by BM-MSCs and we identified the possible target for an anti-inflammatory response.

RESULTS: We demonstrated that BM-MSCs expanded inside the scaffold remain in a stem cell quiescent state mimicking the native environment. Moreover, BM-MSCs expanded inside the Nichoid have a different gene expression profile, compared to controls expanded on plastic, with a significant deregulation of genes involved in cellular pathways including adhesion, morphology, enzymatic activity, and immune response. We confirmed two specific deregulated targets, TNF- α and CSF3, strictly connected to the anti-inflammatory pathways. Finally, a proteomic analysis revealed the effects of the synthetic niche on the secretome of BM-MSCs, modulating the inflammatory response, and controlling the expression of pro- and anti-inflammatory cytokines.

DISCUSSION & CONCLUSIONS: Our findings support a possible clinical translation of BM-MSCs expanded inside the micro-scaffold Nichoid and the secretome as biodrug for the treatment of pediatric pulmonary disorders, including post-COVID lung conditions.

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Keywords: Microenvironment and niche engineering,

OP-032

Layer-by-Layer assembly of ECM based biological macromolecules onto Ti alloy for tackling related bone implant infections

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INTRODUCTION: In the orthopaedic field, implant-associated severe infections rarely occur after complex bone surgeries and cause the dramatic clinical failure of implants. The biofilm formation by antibiotic-resistant microbial pathogens can lead patients to life-threatening complications, as no robust reliable antibiotics may effectively treat the prevention and eradication of biofilms. Hence, there is an urgent need to develop advanced antibacterial coatings on implants to limit the risk of infections. This work focuses on exploiting the nature-inspired self-assembly of biological macromolecules for suppressing biofilm formation while stimulating bone growth.

METHODS: we aim to self-assemble extracellular matrix (ECM) like coatings onto medical-grade titanium (Ti) alloy by exploiting layer-by-layer (LbL) technology. Antibacterial Methylglyoxal (MGO: Manuka honey component) is conjugated with tetra amine-polyethylene glycol (4-NH₂-PEG) to constitute a negative polyelectrolyte. Ours recently patented Hybrid LbL instrument (PCT/GB2020/052641)¹ is exploited to fabricate systematic LbL coatings of type I collagen (COL) and hyaluronic acid (HA) on the Ti alloy surface, incorporating in the top multilayers the antibacterial MGO-g-PEG conjugate. The physicochemical properties of multilayer surfaces were assessed by various analytical techniques such as ATR-FTIR, Profilometry, SEM-EDS, and XPS. The self-assembling process of layers was monitored by contact angle measurements and confirmed the electrostatic interactions by accumulating polyionic constituents.

RESULTS: The cell culture experiments were conducted to prove the osteogenic ability of multilayer coatings using human mesenchymal stem cells by cell viability assay, Live/Dead staining, ALP, and ARS. In addition, we have assessed the antibacterial property of COL/HA/MGO-g-PEG coatings on Ti alloy by quantitative (biofilms formation, CFU) and qualitative (zone of inhibition) methods using *Staphylococcus aureus* and *Escherichia coli* pathogens.

DISCUSSION & CONCLUSIONS: Our study demonstrates that the developed Coll/HA multilayer coatings with the tailor of MGO-g-PEG on covalently immobilized Ti alloy favor the early osteointegration with subside the attack of bacteria in bone tissue engineering

ACKNOWLEDGEMENTS: The authors would like to acknowledge the support of the ORUK fund.

REFERENCES: A.M. Ferreira-Duarte, P. Gentile., Hybrid Layer-by-Layer equipment (patent application PCT/GB2020/052641), 2020.

Keywords: Nanomaterials (inc graphene), Interfaces - engineered

OP-033

Hydrogel Delivery of Liposomal-Vancomycin and DNase I to Eradicate Fracture-related Methicillin-resistant *Staphylococcus aureus* Infection and Support Osteoporotic Fracture Healing

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INTRODUCTION: Fracture-related infection (FRI) is one of the most challenging complications in orthopaedic trauma, which often leads to non-union, permanent function loss and even mortality. Bacterial biofilm formed on implants cannot be easily eradicated by systemic antibiotics, warranting novel treatments. The objective of this study was to investigate the use of a DNase I and Vancomycin delivering hydrogel therapy to eradicate MRSA biofilm and support fracture healing in osteoporotic bone with FRI.

METHODS: Six-month-old Sprague Dawley rats were randomized: (i) ovariectomized (OVX), (ii) ovariectomized+MRSA (OVX-MRSA), (iii) ovariectomized+MRSA+control hydrogel (OVX-MRSA-C), (iv) ovariectomized+MRSA+Vancomycin hydrogel (OVX-MRSA-V), (v) ovariectomized+MRSA+DNase I hydrogel (OVX-MRSA-D), and (vi) ovariectomized+MRSA+DNase I-Vancomycin hydrogel (OVX-MRSA-D-V). Rats undergo ovariectomy-induced osteoporosis (OVX) according to our previously established protocol. A well-established left distal femur metaphyseal fracture model was performed for all rats at 9-months of age. By loading DNase I and vancomycin/liposomal-vancomycin on thermosensitive poly-(DL-lactic acid-co-glycolic acid) (PLGA)-polyethylene glycol (PEG)-PLGA hydrogel, a sequential release of DNase I and vancomycin was achieved. Injection of blank or drug-loaded hydrogel was injected into the fracture site and the wound was closed in layers. Primary outcome was bacteria eradication. Presence of MRSA bacteria in bone tissue was assessed by gram staining, immunofluorescence staining, colony forming unit (CFU), biofilm load, and scanning electron microscopy (SEM). Secondary outcome were fracture healing assessed by X-ray, micro-CT, H&E staining and mechanical testing, and biochemical markers.

RESULTS: Microbiological assessments confirmed bacterial eradication in OVX-MRSA-D-V treatment group. Fracture healing was also rescued in OVX-MRSA-D-V treatment group evidenced by X-ray, Micro-CT, and histology. Mechanical test showed improved ultimate load, stiffness, and energy to failure as well. There was also decreased local expression of inflammatory markers IL-6 and TNF- α , for the OVX-MRSA-D-V treatment group.

DISCUSSION & CONCLUSIONS: In conclusion, we developed a liposomal Vancomycin and DNase I co-delivery hydrogel therapy and validated its therapeutic efficacy in a clinically relevant animal fracture model. The sequential release of DNase I and Vancomycin showed a high degree of biofilm eradication and bacteria clearance, thereby restoring a sterile environment to support osteoporotic fracture healing in FRI.

ACKNOWLEDGEMENTS: This study was supported by the Health and Medical Research Fund (Ref: 19180062).

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Keywords: Drug delivery, Hydrogels and injectable systems

OP-034

Development of a dual-delivery scaffold system containing antibiotic-free antimicrobial nanoparticles and osteogenic nucleic acids for the treatment and regeneration of infected bone tissue

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INTRODUCTION: Regeneration of bone tissue infected by bacteria is one of the greatest challenges of modern tissue engineering(1). Moreover, the increasing problem of antimicrobial resistance, requires the development of solutions alternative to antibiotics. We previously demonstrated that delivering microRNAs from scaffolds can induce endogenous cells to upregulate regenerative genes and proteins with minimal aberrant effects resulting in in vivo healing of bone(2). Building on our expertise, this work develops a dual-delivery scaffold system which simultaneously delivers osteogenic microRNAs (antagomiR-138) and antimicrobial copper-doped bioglass nanoparticles (Cu-BG)(3) with a final aim to develop a platform with superior therapeutic potential which exhibits angiogenic, osteogenic and antimicrobial features.

METHODS: The freeze-dried collagen-based scaffolds containing antagomiR-138 and Cu-BG were assessed in vitro using human mesenchymal stem cells (transfection efficacy, osteogenic and angiogenic gene expression, metabolic activity, DNA content, alkaline phosphatase activity, calcium deposition). The antimicrobial properties of the scaffolds were assessed using gram-positive (*S.Aureus*) and gram-negative (*E.Coli*). The angiogenic and osteogenic properties of the scaffolds were assessed in vivo using a chick chorioallantoic membrane model (CAM).

RESULTS: The CuBG and antagomiR-138 were effectively incorporated into scaffolds to providing osteogenic and antimicrobial features. The scaffolds enhanced the production of reactive oxygen species in *S.Aureus* and *E.Coli* and reduced its adhesion of 5-fold and 2-fold, respectively. The antagomiR138-CuBG scaffolds effectively transfected hMSCs in vitro enhancing the osteogenic (COLL1A2, NOCH4, SMAD5) and angiogenic genes and pathways (VEGFA) and calcium production. When tested in vivo, the antagomiR138-CuBG scaffold promoted vasculogenesis, improving the number of branches and the length of blood vessels in the CAM.

DISCUSSION & CONCLUSIONS: The study showed the great therapeutic potential of a novel, multifunctional biomaterial scaffold developed herein. The scaffold enhanced the expression of multiple osteogenic markers, stimulated the mineralisation of hMSCs, and enhanced vessel formation in CAM. The CuBG nanoparticles enhanced killing mechanisms (production of reactive oxygen species and lipid peroxidation) in *S.Aureus* and *E.Coli* and inhibited the attachment of strains to the scaffolds. Our work demonstrates, for the first time, the feasibility of combining non-antibiotic biomaterial based-approaches with gene therapeutics, which opens the door to a myriad of applications beyond bone repair.

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Keywords: Biomaterials, Infection

OP-035

Tissue Engineered Periosteum for Efficient Delivery of rhBMP-2 and Bone Regeneration

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INTRODUCTION: Despite its unique regenerative capacity, bone healing can be compromised, leading to delayed fracture regeneration and consequently nonunion. Due to the scarcity of autografts and the problems associated with a supraphysiological use of rhBMP-2, novel tissue engineering strategies arise as a promising solution to overcome nonunions and related bone pathologies. Our objective was the fabrication of an engineered periosteum for treating efficiently fracture nonunion and large bone defects generating quality bone tissue.

METHODS: We designed 3D polycaprolactone (PCL) implants composed of an inner cylindric scaffold, produced by PCL extrusion, and an outer PCL membrane printed by melt electrowriting (MEW). While the inner PCL scaffold provides mechanical and structural stability, and an osteoconductive environment, the outer PCL membrane was functionalized with low concentrations of rhBMP-2: BMP(10) (10 µg/ml, 52 ng); BMP(25) (25 µg/ml, 186 ng).

For rhBMP-2 functionalization, MEW membrane was treated with poly ethyl acrylate (PEA) and Fibronectin for efficient rhBMP-2 binding and delivery. The regenerative capacity and therapeutic potential of these scaffolds was tested in vitro for osteoblast differentiation and vivo in a critical size femur defect in Sprague Dawley rats (n≥6 animals/group). Positive (C+, standard treatment, absorbable collagen sponge (+rhBMP2, 186 ng rhBMP-2) and negative controls (C-, non-functionalized implants) were included. Regenerative effects were assessed by qPCR, q-µCT, biomechanical testing and histological as well immunohistological analysis. One way ANOVA or non-parametric Kruskal Wallis test were used for statistical analysis.

RESULTS: In vitro, single loading of 186 ng of rhBMP-2 allows similar differentiation potential over human MSCs that standard osteogenic differentiation medium where fresh rhBMP-2 was added twice weekly, as assessed by alizarin red staining and expression levels of RUNX2, COL1A1, SPP1 and BGLAP.

In vivo, regarding bone regeneration, quantitative µCT analysis shows great bone defect healing and bone formation (BV/TV) for bones treated with rhBMP-2 respect of the C- group. C-, 0.3297±0.0033; C+, 0.5788±0.1235, p=0.8735; BMP(10), 0.7226±0.0906, p=0.0061; BMP(25), 0.9240±0.0966, p<0.0001. When compared with the C+, only BMP(25) group showed significant differences, p=0.0067. After maximum torque normalization with the contralateral intact femur, mechanical testing showed that BMP(25) treated bones were more resistant than C+ and BMP(10) groups: C+, 0.3449±0.2246, p=0.0307; BMP(10), 0.2772±0.2073, p=0.0055; BMP(25), 0.7120±0.2742

DISCUSSION & CONCLUSIONS: In conclusion, PEA functionalized mimetic periosteum show an unprecedented increase in efficiency for bone healing, greatly enhancing rhBMP-2 effects.

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Keywords: Advanced therapy medicinal products, Biologics and growth factors

OP-036

Cisplatin Release from Orthopedic Biomaterials to Minimize Local Recurrence of Bone Tumors After Resection

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INTRODUCTION: Residual tumor cells left in the bone defect after malignant bone tumor resection can result in local tumor recurrence and high mortality. Therefore, ideal bone filling materials should not only aid bone reconstruction or regeneration, but also exert local chemotherapeutic efficacy. However, common bone substitutes used in clinics are barely studied in research for local delivery of chemotherapeutic drugs. Here, we aimed to use facile manufacturing methods to render polymethylmethacrylate (PMMA) cement and ceramic granules suitable for local delivery of cisplatin to limit bone tumor recurrence.

METHODS: Porosity was introduced into PMMA cement by adding 1-4% carboxymethylcellulose (CMC) containing cisplatin, and chemotherapeutic activity was rendered to two types of granules via adsorption. Then, mechanical properties, porosity, morphology, drug release kinetics, ex vivo reconstructive properties of porous PMMA and its in vitro anti-cancer efficacy against osteosarcoma cells were assessed. Morphologies, molecular structures, drug release profiles and in vitro cytostatic effects of two different drug-loaded granules on the proliferation of metastatic bone tumor cells were investigated. Additionally, a rat critical-sized femoral condyle defect model was developed to assess the in vivo biosafety and regenerative capacities of the two different cisplatin-loaded granules. **RESULTS:** The mechanical strengths of PMMA-based cements were sufficient for tibia reconstruction at CMC contents lower than 4% ($\leq 3\%$). The concentrations of released cisplatin (12.1% and 16.6% from PMMA with 3% and 4% CMC, respectively) were sufficient for killing of osteosarcoma cells in vitro, and the fraction of dead cells increased to 91.3% within 7 days. Functionalized xenogeneic granules released 29.5% of cisplatin, but synthetic calcium phosphate granules only released 1.4% of cisplatin over 28 days. The immobilized and released cisplatin retained its anti-cancer efficacy and showed dose-dependent cytostatic effects on the viability of metastatic bone tumor cells. In addition, application of cisplatin-loaded ceramic granules in rat bone defects showed negligible cisplatin accumulation in kidneys and livers at 30 days after implantation. Importantly, no adverse effects on local bone regeneration of the rat femoral condyle defects were observed compared to cisplatin-free controls.

DISCUSSION & CONCLUSIONS: Bone substitutes can be rendered therapeutically active for anticancer efficacy by functionalization with cisplatin without affecting bone regenerative efficacy. As such, our data suggest that cisplatin-loaded bone substitute materials represent viable treatment options for filling bone defects after bone tumor resection.

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Keywords: Drug delivery, Cancer

OP-037

Investigation of Biomimetic Materials "*In Vitro*" and "*In Vivo*" for Engineering Bone Tissue

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INTRODUCTION: Bone healing is scarless and uncomplicated in the majority of cases, however, 5-10% of fractures fail to heal due to the extent of the bone defect, trauma to surrounding tissues or comorbidities such as cancer. This orchestrated healing process relies on various cell types including skeletal stem cells, functional vasculature and growth factors in the microenvironment. The current work examined the biocompatibility and efficacy of innovative, acellular, biodegradable scaffolds, based on an octettruss¹ configuration, coated with biomimetic protein and/or growth factors, to augment bone formation by human bone marrow stromal cells (HBMSCs).

METHODS: Coatings found to be most promising, applied individually or concurrently, were: i) an elastin-like protein (ELP) coating developed at the University of Nottingham² and, ii) a bone-inducing growth factor-based coating of fibronectin (FN) and bone morphogenetic protein-2 (BMP-2), attached to the scaffold by polyethylacrylate (PEA), developed at the University of Glasgow³. Current focus has centred on polycaprolactone (PCL) scaffold compositions as the biocompatible, biodegradable material for clinical translation. HBMSCs were seeded onto PCL scaffolds and alamarBlue™ viability reagent was used to determine cytocompatibility. Thereafter, the chorioallantoic membrane (CAM) assay was used to test biocompatibility prior to the use of murine models.

RESULTS: Material biocompatibility and functionality to induce bone formation by HBMSCs in response to the scaffold material or surrounding coating was confirmed. HBMSCs were seeded onto PCL scaffolds and displayed extensive proliferation on ELP coating and/or growth factor coated PCL scaffolds over a 14-day period indicating excellent cytocompatibility. The chorioallantoic membrane (CAM) assay confirmed biocompatibility of the PCL material, ELP coating and growth factor coating, and the ability of the coatings to support blood vessel formation ($P < 0.05$) in a dynamic biological environment⁴. Optimised coatings applied in a murine subcutaneous implantation model proved inconclusive. The coated PCL material scaffolds were then applied in a murine segmental femur defect model to allow assessment in a limb bone environment.

DISCUSSION & CONCLUSIONS: Novel methods of application of scaffold materials within different in vivo models have been identified with potential for use in future studies, prior to validation in a large animal preclinical bone formation model.

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Keywords: Bone and bone disorders (osteoporosis etc), Biomaterials

OP-038

Gelatin and VEGF incorporation in PVA-Tyramine hydrogels as strategy to enhance vascular infiltration and treat avascular necrosis

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INTRODUCTION: Stimulation of angiogenesis via the delivery of growth factors (GFs) is a promising strategy for the treatment of avascular necrosis (AVN). Tyraminated poly-vinyl-alcohol hydrogels (PVA-Tyr), which have the ability to covalently incorporate GFs, were proposed as a platform for the controlled delivery of GFs to the necrotic areas [1]. Nevertheless, the hydrophilicity and bioinertness of PVA limits its integration with the host tissues. The aim of this study was to investigate the effectiveness of incorporating gelatin, an FDA-approved, non-immunogenic biomaterial with biological recognition sites, as a strategy to impart biofunctionality to PVA-Tyr hydrogels to promote vascular ingrowth to necrotic tissues.

METHODS: Progressively higher gelatin concentrations (0.01-5wt%) were incorporated in the PVA-Tyr network. Hydrogel physico-chemical properties and endothelial cell attachment were evaluated. Vascular endothelial growth factor (VEGF) was selected as the angiogenic GF of choice for this study. The controlled release of VEGF from the PVA-Tyr-gelatin hydrogel and vascular functionality was evaluated using immunofluorescence quantification and chorioallantoic membrane (CAM) assay. VEGF-loaded PVA-Tyr hydrogels with or without gelatin (n=7) were implanted in a subcutaneous mouse model for 3 weeks. Vascularization (CD31+ cells) and cell infiltration (H&E) were evaluated. Finally, AVN was induced in 6 weeks old male piglets as previously described [2]. A transphyseal hole (3mm) was drilled and PVA-Tyr hydrogels with 1% gelatin were delivered in the defects. Piglets were euthanized after 4 weeks and microCT analysis was performed.

RESULTS: The incorporation of 1% gelatin significantly enhanced cell attachment without compromising hydrogels physical properties, degradation, VEGF retention and release. Thus, this gelatin concentration was selected for further analysis. Additionally, VEGF that was covalently incorporated into the PVA-Tyr-gelatin network retained its bioactivity, and successfully promoted neo-angiogenesis in a CAM assay. Interestingly, the subcutaneous implantation studies showed that incorporation of gelatin into PVA-Tyr hydrogels did not increase cellular infiltration or interaction when compared to PVA-Tyr only hydrogels. However, the VEGF-loaded PVA-Tyr-gelatin hydrogels did facilitate higher vascular infiltration into the hydrogels. Additionally, preliminary microCT results indicated that the delivery of these biosynthetic hydrogels in an AVN model was effective in preventing the necrosis-associated resorption of the bone.

DISCUSSION & CONCLUSIONS: These results indicated that the biofunctionality of PVA-Tyr hydrogels can be improved via incorporation of gelatin. Additionally, preliminary results suggested the suitability of the developed hydrogels to treat AVN.

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Keywords: Hydrogels and injectable systems, Bone and bone disorders (osteoporosis etc)

OP-039

An innovative 3D bioengineered model as a tool to elucidate the role of human dermal ECM and immune cells in wound healing and scar formation processes

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INTRODUCTION: The skin isolates and protects the body from external agents, and in case of injury, it initiates a natural biological phenomenon, wound healing, during which the body blocks the bleeding, heals and closes the wound.

The entire complex biological process is strongly influenced and regulated by the inflammatory response triggered by both tissue-resident and circulating cells of the immune system. Although inflammation occurs almost immediately after the injury, it strictly regulates the final stages of healing. In particular, the mediators released by activated immune cells stimulate fibroblasts towards the production of scar tissue and, consequently, give shape to the final repair result.

Most of the wound healing experiments are based on a 2D culture of cells, mainly epithelial cells and fibroblasts.

Despite the advantages and ease-of-use for the study of cell migration and proliferation, essential for understanding the processes of angiogenesis and re-epithelialization, in vitro 2D assays cannot summarize the complex events underlying wound healing phenomenon.

Instead, tissue engineering aims to reproduce a functional and structural dermal substitute by creating three-dimensional scaffolds as an extracellular matrix (ECM) analog to guide cell adhesion, growth, and differentiation.

METHODS: In this perspective, the complexity of this biological process can be modeled with a device capable of hosting a 3D human dermal equivalent (3D-HDE), produced with a bottom-up approach. The HDE is composed of human fibroblasts embedded in their own extracellular matrix. After inducing a second intention wound through a 1 mm-biopsy punch, macrophages responsible for the induction of scar tissue formation are subsequently added.

RESULTS: The endogenous model used in this work has shown the ability to replicate crucial in vitro phenomena related to the restoration and remodeling of ECM after causing a wound: (i) fibroblast activation and interaction with ECM (ii) ECM remodeling, assembly and turnover, (iii) activation of MMPs of the ECM.

In addition, the model proposed will be used to explore the role of immune cells in the wound.

Preliminary results have shown how these endogenous matrices can interact with macrophages once

they have been introduced after injury.

DISCUSSION & CONCLUSIONS: This immune-HDE would significantly and remarkably increase the knowledge of the wound healing process and scar formation. Working in this direction, this immunocompetent endogenous model can faithfully emulate the human trauma response, highlighting the role of macrophage-released TGF- β 1 and the subsequent expression of α -SMA and collagen I deposition.

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Keywords: Skin, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-040

The hurdles of collagen shrinkage: A promising GelMA-based organotypic skin approach

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INTRODUCTION: Collagen I (Col I) is the gold standard material to generate many in vitro tissue models, including organotypic skin. Col I remodeling by fibroblasts incorporated in the dermal part of the model leads to significant dimensional changes associated with additional hurdles when cultured within dynamic culture systems. These systems are relevant for the biofunctionality mimicry of the skin due to its multilayered nature and by providing the necessary fluid flow conditioning. Gelatin methacrylate (GelMA) is proposed in this study to validate a dimensionally stable organotypic skin model developed and maintained in a custom-made bioreactor [1], generating a dynamic in vitro testing platform.

METHODS: Human dermal fibroblasts (hdFBs)-laden 7.5% GelMA hydrogels were prepared with a 2.3×10^5 cell/ml density and crosslinked for 30 seconds under 7.2 mW/cm² UV light. After 14 days in culture, uniaxial compression test and dynamic oscillatory tests were performed. Mechanical properties were compared with standard Col I-based hydrogels. The organotypic skin model was prepared by coating a 0.26% Col I solution onto the GelMA hydrogels immediately after the encapsulation of hdFBs. The Col I was let to polymerize for 1 hour at 37°C and the whole construct was cultured for 7 days. After this, 5×10^4 keratinocytes (KCs) were seeded on top, and constructs were cultured for more 7 days before being lifted to air-liquid interface (ALI) for KCs stratification. The model was characterized histologically regarding cell differentiation (epidermis), basement membrane (dermal-epidermal interface) and dermal extracellular matrix deposition.

RESULTS: The storage modulus of GelMA presented a mean value of 1kPa throughout the culture, whereas the mean value of Col I, increased from 0.5 to 1.2kPa. Likewise, the compression modulus of GelMA of 18kPa was maintained, in contrast to the 2-fold stiffer value of Col I at day 14. The shrinkage ratio of GelMA and Col I was circa 20% and 83%, respectively. The obtained organotypic model was characterized by a fully differentiated epidermis and extensive proliferation of hdFBs coupled with significant Col I and Fibronectin deposition.

DISCUSSION & CONCLUSIONS: So far, the generated GelMA-based organotypic skin seems to be a suitable candidate for surpassing the struggles associated with collagen shrinkage observed in the standard organotypic skin model. Future experiments will focus on the dynamic culture of the skin equivalent in the recently developed sandwich-like bioreactor.

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Keywords: In vitro microenvironments, Skin

OP-041

Bioengineered human skin model to study multiple aging-related phenomena in vitro

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INTRODUCTION: Skin aging has always attracted a lot of interest from a research point of view, as skin represents the first barrier between human body and the surrounding environment. Among the various exogenous stress factors affecting human skin over lifetime, UV radiation and accumulation of advanced glycation end-products (AGEs) represent phenomena raising a lot of concern. Indeed, skin is continuously exposed to UV light, thus leading to a continuous damage to both cells and extracellular matrix (ECM). AGEs are a result of non-enzymatic glycation of the ECM proteins occurring after long-term exposure to sugars. Moreover, dietary habits can greatly influence the level of AGEs in human body, resulting in a progressive impairment of cells and ECM function.

METHODS: To study the effects of different aging-related phenomena, a full thickness human skin equivalent (HSE) comprising a complex human dermal equivalent (HDE) is produced. The HDE obtained is characterized by the presence of human dermal fibroblasts embedded in their own ECM, thus giving the model the ability to faithfully recapitulate structure and function of the human skin in pathophysiological conditions. Long-term UV exposure will be simulated through multiple expositions to an UV lamp, with parameters such as exposure time and irradiance accurately chosen to simulate a lifetime damage. AGEs accumulation will instead be induced by treating HDEs with glyoxal, a highly reactive intermediate compound in the formation of glycation products. Morphological changes can be quantified through H&E staining, while the progression of the inflammatory state typical of both phenomena can be monitored through Elisa kit analysis. Moreover, immunofluorescence analyses with AGEs-specific antibodies can be adopted. Finally, mechanical properties of the reconstructed skin samples can be assessed through microindentation techniques.

RESULTS: Multiple exposure to UV rays leads to a progressive decrease in cell number as well as a destruction of ECM structure. This is accompanied by a progressive raise in reactive oxygen species (ROS) level, as expected when dealing with UV radiation. The accumulation of AGEs modifies the organization of collagen fibers, also leading to profound changes in mechanical properties of the samples. Furthermore, the onset of an inflammatory response deeply affects cells and tissue homeostasis.

DISCUSSION & CONCLUSIONS: Our work demonstrates that the proposed endogenous skin model is a

suitable platform to investigate – at both cellular and extracellular level – complex aging-related phenomena. As further development, such platform can be used as an alternative to animal testing to assess the efficacy of anti-aging compounds.

Keywords: In vitro microenvironments, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-042

Mechanically tuneable, suspended layer additive manufactured 3D-printed cell-laden pectin matrices with PODS® microcrystal biochemical functionalisation for skin modelling

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INTRODUCTION: To successfully recapitulate their native counterparts, human tissue models need to incorporate a multitude of factors. Beyond the appropriate cell types, cell attachment to the bioscaffold, the bioscaffold's mechanical properties, and the continuous exposure to growth factors and cytokines, all contribute to how representative a model is. Previously, our group has shown that Suspended Layer Additive Manufacture (SLAM) 3D bioprinting can yield sequential skin layers with different cell types and densities. In the present work we explored a method for individual biochemical functionalisation of each tissue layer, that can be sustained over weeks. Used were polyhedrin-based slow-release PODS® microcrystals, loaded with different growth factors or cytokines. The work also has begun to define the range of mechanical properties that can be engineered into layers, using a CaCl₂-crosslinked pectin biomaterial.

METHODS: The models were printed with a cell-laden low-methoxyl pectin solution in an 0.5% w/v agarose suspension bath, subsequently crosslinked with CaCl₂(aq) solution. Differential biochemical functionalisation was achieved by dispersing specific PODS microcrystals through the bioink of each layer. The mechanical and viscoelastic properties were determined based on loss and storage moduli, obtained via dynamic mechanical analysis on a mechanical compression tester. Release kinetics data for the PODS® microcrystals was derived from enzyme-linked immunosorbent assays (ELISAs).

RESULTS: The SLAM technology allows printing Human Dermal Fibroblasts (HDFs) – the primary dermal cell type – with over-75% viability, maintained over 40+ days. Mechanical analysis of the gels shows that varying the combination of polymer and crosslinker can modulate the ultimate stiffness and the viscoelastic properties of the 3D printed models. Analysis using ELISA of conditioned media samples in which PODS-laden gels have been incubated indicated that release of biologically relevant molecules, such as IL-6, can be maintained in excess of 3 weeks.

DISCUSSION & CONCLUSIONS: By incorporating long-term viability of HDFs, PODS microcrystals, and wide-ranging mechanical properties profile of the bioscaffold, the present 3D printing platform exhibits potential for recapitulating multiple states of the dermis, i.e. in healthy skin, wounds, and scars. Previous research from our group also indicated that co-culture with Human Epidermal Keratinocytes (HEKs) and Adipose Derived Stem Cells (ADSCs) is possible and we intend to investigate this additional complexity in the model. Combined with the novel mechanical and biochemical aspects introduced to the SLAM 3D bioprinting platform, these findings expand our models' potential to recapitulate skin conditions for the purposes of research, pre-clinical testing, and even for tissue replacement therapies.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Biologics and growth factors

OP-043

Reducing wound infection with novel antimicrobial treatments in human *ex vivo* and porcine *in vivo* wound models

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INTRODUCTION: Open wounds disrupt the barrier function of the skin and increase risks of infection. The current threat of antibiotic resistance coupled with slow development of novel antimicrobial treatments calls for new therapeutic options as well as new methods for evaluation of potential therapeutics. The aim of the present study was to develop a standardized infected human *ex vivo* wound model for rapid screening of novel treatments and to evaluate the antimicrobial effects of the antimicrobial peptide (AMP) Plantaricin NC8 $\alpha\beta$ (PLNC8 $\alpha\beta$) using human *ex vivo* and porcine *in vivo* models.

METHODS: The *ex vivo* wound model was based on skin from healthy patients undergoing plastic surgery (Swedish Ethical Review Authority approval: 2018/97-31). Circular full-thickness biopsies (8 mm diameter) with a central partial-thickness wound (4 mm diameter) were created using biopsy punches. Wounds were inoculated with 10^9 *Staphylococcus aureus* (ATCC 29213) and maintained under standard culture conditions up to 14 days. The *in vivo* wound model was conducted on four female pigs for ten days (Regional Animal Ethics Board approval: ID1418). *S. aureus* inoculated wounds were treated with topical administration (100 and 300 μ M) of both D and L-enantiomers of the AMP PLNC8 $\alpha\beta$. Moreover, topical gentamicin administration (3 μ g/ml-10 μ g/ml) was evaluated regarding eradication of infection.

Tissue infection was monitored using quantitative cultures on selective media as well as immunofluorescent staining using primary antibodies directed towards *S. aureus*. Moreover, reepithelialization was evaluated using routine histology and immunofluorescent staining for pancytokeratin.

RESULTS: Quantitative cultures revealed stable wound inoculation of approximately 10^{7-8} colony forming units (CFU)/g of tissue without antimicrobial treatment. Immunofluorescent staining further confirmed presence of *S. aureus*, reaching through the dermal layer. Reepithelialization was not observed in infected wounds 10-14 days post inoculation, whereas non-infected control wounds were covered with neoepidermis. Topical treatment with PLNC8 $\alpha\beta$ reduced wound infection below the clinical relevance of 10^5 CFU/g. Combinational therapy with gentamicin completely eradicated infection seven days post inoculation.

DISCUSSION & CONCLUSIONS: The current study describes the establishment of a reproducible and standardized infected *ex vivo* wound model, which provides rapid screening of novel antimicrobial treatments. Furthermore, it presents a promising antimicrobial activity of the AMP PLNC8 $\alpha\beta$ against *S. aureus* in both human *ex vivo* and porcine *in vivo* wounds. In addition, enhanced efficacy of antibiotics administered together with the AMP may enable lower doses of antibiotics, reducing resistance development.

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Keywords: Infection, Skin

OP-044

Various skin test systems and their applications: From epidermis to full thickness skin to hiPSC derived skin organoids

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INTRODUCTION: In vitro skin models offer fast and reliable investigations to develop therapeutic interventions and the possibility to study diseases of the skin. The existing test systems can be matched to the questions due to their complexity. Classical 2D-cell culture is very fast and offers the possibility of rapid testing in high throughput, due to the complexity of human skin they are partly representative.

METHODS: In our lab we use reconstructed epidermis (RHE) to investigate the function of the epidermal part of human skin. These RHE cultivated in an air-liquid interphase form a basal layer consisting of keratinocytes. They form a 3D structure leading to a barrier function comparable to human epidermis. Even more complexity offers three-dimensional full-thickness skin equivalents (FTSE). Therefore, fibroblasts are combined with a collagen matrix to mimic dermis with keratinocytes seeded on top to form a functional epidermis cultivated also in an air-liquid interphase. To expand our in vitro skin test-system line-up, we use hiPSC derived skin organoids, which mimic complex skin tissue.

RESULTS: The RHE are used for physiological and pathological questions. RHE serve as a perfect tool to measure the barrier function of the skin and with integrated melanocytes for investigation of pigmented skin. Keratinocytes from patients with epidermolysis bullosa simplex can also be extracted and used as an in vitro test system. In addition, RHE is used to investigate epidermal burn wounds. RHE and FTSE are for use in wound healing experiments. More complex test systems are from hiPSC derived skin organoids, mimicking the architecture of human skin with its hair follicle and appendages. Applications here include skin fibrosis, skin cancer and infection studies.

DISCUSSION & CONCLUSIONS: The various test systems have their different strengths and applications and enable to investigate skin function, test drugs and understand the mechanism of skin defects and skin regeneration.

Keywords: Induced pluripotent stem cells, Disease models

OP-045

Assessment of the Impact of Antibiotics against *Pseudomonas aeruginosa* on Human Skin Cell Lines and Bioengineered Autologous Skin Substitutes: an *in vitro* Study

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INTRODUCTION: Loss of skin integrity due to injury can cause physiologic imbalance and ultimately significant disability or even death. In the case of severe injuries, the self-healing capacity of skin is insufficient and the wound healing process can be compromised. Approaches to improve wound healing include the administration of autologous skin cell suspensions and the development of bioengineered autologous skin substitutes (BASS). In patients with severe injuries, antibiotic or antiseptic treatments are crucial to prevent and treat infections. Antiseptics have been reported to be cytotoxic to skin cell lines and BASS at clinical concentrations. However, few studies evaluate the impact of antibiotics on these cells and skin substitutes. The aims of this study were to evaluate the impact of antibiotics against *Pseudomonas aeruginosa* on human fibroblasts (HFs) and keratinocytes (hKTs) and on BASS and to determine the concentrations that maintain their viability and functionality while preserving its antibacterial capacity.

METHODS: Amikacin, ciprofloxacin, colistin and meropenem cytotoxicity was evaluated on HFs and hKTs and on BASS. Control groups were untreated. Cell viability (Live/Dead®), cell proliferation (AlamarBlue™) and cytokine secretion (ELISA) were performed on cell monolayers after antibiotic treatments. Cell migration capacity was also evaluated (Wound Closure Assay). For BASS, hKTs and HFs were isolated from skin samples and integrated into a fibrin- hyaluronic acid scaffold. Regarding the impact of treatments on BASS, cell viability (Live/Dead®) and skin barrier integrity (Transepidermal Water Loss, TEWL) were evaluated.

RESULTS: All treatments maintained HFs proliferation similar to the control. Cell viability was also not affected. For hKTs, amikacin and colistin significantly reduced cell proliferation and viability comparing to ciprofloxacin and meropenem. Cell migration was not affected but the secretion of

cytokines related with the wound healing process was altered after antibiotic treatments in both cell monolayers. Regarding BASS viability, no significant differences were observed between treatments and control. The skin barrier function was also conserved after antibiotics treatments and TEWL measurement was reduced at day 14 of follow-up when BASS were cultured in an air-liquid interface, reflecting a better epithelium development.

DISCUSSION & CONCLUSIONS: This study revealed the maximum concentration of these antibiotics which preserved HFs, hKT and BASS viability and functionality but also has antibacterial capacity against *Pseudomonas aeruginosa*. These findings set the basis for future studies that aim to develop BASS- containing antibiotics and also show how these antibiotics can affect therapies currently used in wound care, such as the administration of skin cells suspensions.

Keywords: Infection, Wound healing

OP-046

Towards the Engineered Chondron: Pericellular Matrix Synthesis by Articular Cartilage Chondrocytes and Chondroprogenitors in Agarose Microgels

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INTRODUCTION: In cartilage tissue engineering, chondrocytes are often encapsulated in bulk hydrogels at high densities. However, since mass transfer in bulk hydrogels is poor, and chondrocyte expansion while maintaining phenotype is limited, alternative methods should be explored. Microfluidics produced cell-laden hydrogel microgels are presented as a system to overcome the mass transfer limitations of bulk hydrogels. By exploiting these droplets, this study aims to compare pericellular matrix (PCM) production, a hallmark of chondrogenic characteristic phenotype and the necessary first step in cartilage regeneration, by primary chondrocytes and chondroprogenitors, a potential alternative cell source with increased expansion capabilities.

METHODS: Chondrocytes were isolated from bovine articular cartilage. From this population, chondroprogenitors were isolated using differential adhesion to fibronectin. A PDMS droplet microfluidic device was used for the encapsulation of the cells in 1% (w/v) agarose microgels. Microscopy was used to determine cell distribution and microgel size. Microgels were cultured with chondrogenic medium. Chondrocytes were stimulated with TGF- β +dexamethasone. Chondroprogenitors were provided with pulse stimulation of BMP-9 followed by TGF- β +dexamethasone stimulation. Cell viability was assessed with DAPI using flow cytometry. Matrix synthesis was assessed with immunostaining for collagen type-I/type-II/type-VI using flow cytometry and fluorescence microscopy.

RESULTS: Primary chondrocytes and chondroprogenitors were successfully encapsulated in agarose microgels. Microscopic observation showed cell-laden microgels mainly contained one cell per droplet with an average diameter of 54 μ m. Chondrocytes were viable after encapsulation and remained metabolically active for at least 21 days. Flow cytometry showed that 97% of cells were positive for collagen type-VI after 7 days. While absent at day 1, compact rings of collagen type-VI around cells were observed with microscopy at day 7 and day 21. Additionally, collagen type-II synthesis increased during culture and its presence coincided with collagen type-VI. Collagen type-I remained consistently low over 21 days.

DISCUSSION & CONCLUSIONS: Using the presented method, chondrocytes and chondroprogenitors can be encapsulated in agarose microgels and matrix production can be analyzed. The chondrogenic phenotype was maintained as newly synthesized collagen type-VI was present, collagen type-II increased, and collagen type-I remained stable. Further analysis and comparison between chondrocytes and chondroprogenitors are pending. This method provides high control over the cellular microenvironment and could have great impact on improving cartilage tissue engineering approaches.

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Keywords: Cartilage / joint and arthritic conditions, In vitro microenvironments

OP-047

Bicompartmental culture system hosting different substrates to enhance the biomimicry of in vitro barrier models

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INTRODUCTION: The intestinal epithelium, which consists mostly of an enterocytic monolayer, forms a physical barrier that is pivotal in the molecular absorption process. Despite the need for complex intestinal in vitro model to carry out predictive and reliable pharmacological investigations, standard cell cultures are mainly performed using plastic materials which are not functionalized and fail in replicating the micro-physiological environment, while the proper culture substrate is crucial to enhance the biomimicry of the model and its predictive potential [1]. To overcome these limitations, we developed a bicompartmental versatile culture system, named True Tissue on Platform (TTOP) which allows to host tunable culture substrates, such as microporous membranes or scaffolds, in a scalable and cost-effective manner.

METHODS: To demonstrate the dependency of cellular behavior on the culture substrate, we carried out a comparative study by using Caco-2 cell lines cultured on bare polycarbonate porous membranes, coated membranes (gelatin, fibronectin, collagen) and various types of scaffolds, such as different types of electrospun gelatin scaffolds – consisting of crosslinked mixture of gelatin and resilin peptides– and silk-based electrospun scaffolds. Caco-2 growth rate has been monitored through Trans Epithelial Electric Resistance (TEER) measurements and live imaging, while Immunofluorescence (IF) endpoint assays evaluated the differentiation by staining tight junctions and specific membrane proteins (ZO-1, Villin, Phalloidin), and the three-dimensional architecture, thanks to Z-stack imaging.

RESULTS: The results showed that the functionalization of the culture substrate plays a fundamental role in the formation and tightness of the gut barrier. Indeed, with respect to the cells cultured on bare membranes, the coated samples and the scaffold-based cultures reached a monolayer earlier. This was confirmed also by TEER measures at different time points. Moreover, IF staining demonstrated that the substrate properties promote differentiation and the three-dimensional cellular organization.

DISCUSSION & CONCLUSIONS: In conclusion, the versatility of TTOP enabled to carry out comparative studies with different culture substrates using the same platform. This will allow to specifically study the effects of the extracellular environment on cellular architecture and differentiation, without artifacts introduced by the use of different devices and equipment. Since the biological results demonstrated that the substrate's features promote differentiation and enhance the formation of three-dimensional cellular constructs, TTOP scalability and ease of use with complex substrates could facilitate the development of more predictive in vitro barrier models.

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Keywords: In vitro microenvironments, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-048

Enhancing neuronal cell network organization and activity using NOA81 and PDMS nanogrooves

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INTRODUCTION: Nanoscale topographies, e.g. nanogrooves, influence differentiation and maturation processes in cultured neuronal cell networks [1-4]. Multi-electrode arrays (MEA) can be used to assay the effect such surface modifications have on neuronal activity. This work revisits culture experiments with polydimethylsiloxane (PDMS) nanogrooves [5] and presents nanogroove-integrated MEA plates for their utilization in nervous system-on-chip (NoC) research [6].

METHODS: To assess whether enteric nervous system (ENS) cells can grow on replica-molded nanogrooves and in NoC devices, first, WTC-11 induced pluripotent stem cells (iPSCs) were differentiated toward enteric neurons and glia [7] and then re-plated and cultured within various PDMS chip configurations. To fabricate Norland Optical Adhesive 81 (NOA81) nanogrooves on MEA, microtransfer molding [8] and UV lithography was used, which allows to access the electrodes during cell culture. We used Ngn2+ iPSC-derived neurons [9] to assay neuronal activity. These cell experiments were performed on bare as well as nanogroove and flat NOA81-modified MEAs. A MEA-2100 reader was used to record at day 18 and MEA plates were also subjected to immunocytochemistry on day 21 to confirm the neuronal identity and to visualize the axons.

RESULTS: Our observations demonstrated that WTC-11 iPSC-derived ENS progenitors and enteric neurons can differentiate and survive well for up to 30 days on PDMS devices either with or without nanogrooves. With regard to electrophysiological assessment of the influence of NOA81 on MEA, both the flat polymer layer and the nanogrooves showed a significantly higher number of spikes compared to the bare MEA plates. Nanogrooves showed also 21% higher spike activity than flat NOA81. Immunostaining by SMI 312, which marks neuronal axons, also confirmed alignment of neuronal outgrowth to the direction of the nanogroove pattern upon analyzing the images by Fiji Directionality Analysis [10].

DISCUSSION & CONCLUSIONS: We successfully performed ENS cultures in PDMS microfluidic devices with connected compartments and fabricated NOA81 nanogrooves on MEA plates. Ngn2-based cortical readout system on MEA confirmed that nanogrooves influence the network formation and activity. Next, ENS cells will be incorporated in connective compartments with Central Nervous systems cells in order to close the neural circuitry of a Nervous System on Chip designed on MEAs.

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Keywords: Nervous system (brain-central-peripheral / disorders), Biomechanics / biophysical stimuli and mechanotransduction

OP-049

3D bioprinted cancer model for in vitro drug testing

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INTRODUCTION: Chemotherapy has been widely used as a cancer treatment option. However, this therapeutic approach is still largely palliative or causes severe side effects to date. Therefore, the development of new anti-cancer drugs remains an urgent need. Traditional research platforms, such as two-dimensional (2D) culture and animal trials, differ from natural human cancer tissues in many aspects, resulting in fortuity and unreliability. Inspiringly, three-dimensional (3D) bioprinting has been emerging as an efficient technique to produce in vitro human-derived 3D models with mimicked natural tissue structure and recapitulated tumor microenvironment. It shows great potential to facilitate the research of anti-cancer drugs.

METHODS: In this study, a BioX 3D bioprinter (Cellink) was used to produce neuroblastoma renal cancer models, consisting of neuroblastoma, human embryonic kidney 293 cells or primary human kidney fibroblasts. Cell viability was measured by XTT and Live/Dead assays after treatment with either panobinostat or blasticidin. Apoptosis was evaluated by staining of cleaved caspas-3. In addition, a perfusable 3D model of neuroblastoma hepatic cancer was fabricated using a 3D discovery bioprinter (regenHU). Inside the stromal hepatic part, there was a neuroblastoma cancer core surrounded by perfusable channels. The media, containing the respective anti-cancer drug, were perfused through the models and cell response was evaluated afterwards.

RESULTS: In the neuroblastoma renal cancer model, panobinostat selectively killed the cancer cells by apoptosis induction but did not affect renal cells in the therapeutically effective concentration range. In contrast, blasticidin induced cell death in both cell types. Importantly, differences in sensitivity between 2D and 3D cultures were cell-type specific, making the therapeutic window broader in the bioprinted model [1]. Additionally, a perfusable neuroblastoma hepatic cancer model was successfully fabricated. Meanwhile, a highly-adaptable perfusion setup was developed as well. Here the cells in the perfused models showed a higher viability than those in the submerged ones. The growth of the encapsulated cancer part was inhibited by the perfusion with the anti-cancer-drug containing media, while the stromal region was not severely affected.

DISCUSSION & CONCLUSIONS: Neuroblastoma renal and hepatic cancer models developed by 3D bioprinting methods can be used to study the interaction between the tumor area and stromal tissue, and to examine the anti-cancer effect of drugs. It demonstrates the value of studying anti-cancer drugs in human 3D models, and also indicates that bioprinted models can be valuable for therapeutic studies of other diseases.

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Keywords: 3D printing and bioprinting, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-050

3D-printed cell culture system as an in vitro platform for non-small cell lung cancer (NSCLC) modeling

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INTRODUCTION: Lung cancer is a heterogeneous disease and represents the leading cause of cancer-related death (1). Progression is promoted by a complex microenvironment, which includes several cell types, matrix components (2) and pervasive angiogenesis (3). Since traditional 2D culture fails at resuming the complexity of in-vivo model, the aim of this work is the development of a printed in-vitro device, that mimic NSCLC, to be used as platform for molecular studies and drug screening.

METHODS: Hydrogels based on methacrylated gelatin (GelMA) (4) were used as matrix. To mimic NSCLC, adenocarcinoma cell lines, A549 and H1299, and normal fibroblasts, MRC-5, were selected. Then, biocompatibility of different formulations were analyzed by MTT assay, Calcein AM/PI staining and flow-cytometry. Matrix production was assessed by qRT-PCR, western blot and immunofluorescence analysis. For 3D printing, an extrusion-based system (Regenhu) was used.

RESULTS: Three different formulations (Medium, High, Very High), were obtained by modulating gelatin degree of functionalization. These matrices were tested at different concentrations to identify preferable culture conditions. A549 and H1299 were grown as mono-culture or co-cultures at two tumor:fibroblast ratios (1:1 and 1:2), to investigate the cross-talk between these two populations. MTT analysis revealed promising proliferation increase in High and Very High, both at 10%, for A549 model; whereas, for H1299, GelMA percentage must be increased at 12,5%. Absence of matrix toxicity was confirmed by Calcein/PI staining. Moreover, the maintenance of the ratio of the two cell types was verified by flow-cytometry. Protein and RNA analysis showed fibroblast activation in terms of α -SMA and Collagen I expression when cultured in 3D, whereas this modulation was lost in the co-cultures suggesting an interplay between the two populations. Further, both models were successfully printed by extrusion without affecting viability. Printing optimization allowed the designing of channels inside the scaffold to mimic vasculature contribution.

DISCUSSION & CONCLUSIONS: Our results demonstrate the feasibility of using GelMA as matrix for 3D long term cell culture to study the interplay that occurs between different components of the tumor microenvironment. Further, GelMA printability allow the implementation of the model by adding a microfluidic system inside the scaffold to mimic the physiological vasculature and investigate endothelium contribution.

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Keywords: Biomaterials, Cancer

OP-051

Influence of cell viability assay on cellular metabolisms

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INTRODUCTION: The well-known cell viability test conducted by water-soluble tetrazolium salt (WST-8) has been widely used for biomaterials research to assess cell viability and cytotoxicity. WST-8 (also called CCK-8) is frequently utilized as an endpoint test in tissue engineering fields. However, CCK-8 can also be applied to a pre-measurement procedure. It is also suggested by technical manuals that "cells can be further used for other experiments."

However, cells with similar viability do not necessarily represent a similar biochemical state. Until now, the intracellular changes of the assay-treated cells are not well studied and often overlooked. Here we raised a very practical question: is continuous usage of the same cells after cell viability assay possible? Here, we relied on metabolomics as it provides readouts closest to phenotype and cellular metabolic adaptations are faster than other biomolecules, e.g., genes and proteins.

METHODS: We collected intracellular metabolites from WST-8 treated cells (NIH/3T3 cell line) and used the LC-MS-based method to analyze the metabolic profile. The WST-8 treated cells were also imaged by live/dead staining and compared against the control. The data were analyzed using cluster analysis, student t-test, and pathway analysis.

RESULTS: The staining images showed no differences between treated and untreated cells. However, the metabolic profiles were significantly changed compared to controls. PCA score plots were separated explicitly into two clusters. Uridine was downregulated considerably by WST-8, while adenosine, succinic acid, glucose 6-phosphate, aspartic acid, palmitoylcarnitine, and butyrylcarnitine were upregulated. Pathway analysis revealed influenced metabolism pathways, of which the TCA cycle, propanoate metabolism, fatty acid degradation, and propanoate metabolism were the most significantly impacted.

DISCUSSION & CONCLUSIONS: The mostly affected metabolites play significant roles in the TCA cycle, glycolysis, and carnitine biosynthesis. We confirmed that, although from a macro perspective, WST-8 is considered to be with very low toxicity and displays no difference in live/dead imaging, the intracellular metabolisms were significantly altered. The influenced pathways were mainly in energy metabolism, which is highly related to NADH and NADPH. We recommend that researchers avoid using the WST-8 treated cells for other experiments based on our findings. At the same time, we demonstrated that metabolomics is a powerful tool that can provide a deeper understanding of cellular status.

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Keywords: Omics / bioinformatics and systems biology, Innovation

OP-052

Understanding the behaviour of Notochordal cells in healthy and degenerated disc environment and their anti-catabolic properties on degenerated Nucleus pulposus cells

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INTRODUCTION: Low back pain associated with intervertebral disc (IVD) degeneration is a socioeconomic problem. Current treatments are symptomatic and involve invasive surgeries, whilst there are several therapies under investigation including cell-based strategies. Here, we developed media which more closely mimics the low glucose, pH and OSM seen in the disc niche. These media were then utilised to investigate the behaviour of porcine notochordal cells (pNC) with degenerated human NP cells to further understand pNC anti-catabolic effect on degeneration process.

METHODS: Human NP cells isolated from patients undergoing microdiscectomy surgery and pNCs, isolated from porcine spine encapsulated in 1.2% alginate beads were cultured in Standard (400 mOsm/kg), healthy 450 mOsm/Kg, pH:7.1) and degenerated (350 mOsm/kg, pH:6.8) media $\pm$ 100pg/ml IL-1 β that mimic the native disc. The culture of human NP cells was established either alone or in co-culture with pNCs or stimulated with notochordal cell condition media (NCCM). Cells were sampled after 72 hrs for gene expression and Luminex analysis, and 1 week for Digiwest analysis.

RESULTS: Comparing human NP cells cultured in different healthy and degenerated disc niche showed significant higher secretion of cytokines, chemokines and matrix degrading enzymes in degenerated+ IL-1 β media compared to other treatments. Digiwest analysis showed increased protein production for Cytokeratin 18, Src and Phosphorylated PKC but a decrease in fibronectin in degenerated media compared to standard media. qPCR analysis showed significant decrease in VEGF, MMP3 and increase in COL II in NC treated cell. Co-culture of NP cells and pNC cells showed significant increase in IL-8 expression in healthy in NP+NCCM group compared to NP cells alone.

DISCUSSION & CONCLUSIONS: Development of media to mimic the healthy and degenerate niche has enabled the study of NC and NP cell behaviour in vitro in an environment closer to the disc niche. The disc environment affects how cells behave in terms of their phenotypic markers and catabolic responses. Highlighting the importance of designing the in vitro systems as close as possible to native disc environment particularly for testing and designing new cell-based treatments for disc degeneration-associated low back pain. This work is being translated to investigate the potential use of iPSCs differentiated into notochordal like cells as potential regenerative cell sources.

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Keywords: Cell therapy, Other

OP-053

The pro-repair effects of tissue-derived extracellular vesicles are modified with ageing

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INTRODUCTION: The risk of many lung diseases increases considerably with age, these include chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Currently, there are no curative treatments available for age-related lung diseases. The lungs have significant intrinsic repair capacity but this declines with age. Extracellular vesicles (EVs) are cell-secreted nanoparticles that are likely to play a key part of intrinsic repair through the transfer of biological cargo including signalling proteins, nucleic acids and lipids. To date EV research has focused on mesenchymal stem/stromal cell-derived EVs but tissue-derived EVs are likely to contain cargo that is specialised/enriched for repair of their tissue of origin. We have characterised lung tissue-derived EVs for the first time and investigated their capacity to repair injured lung tissue from young and aged mice.

METHODS: Precision-cut lung slices (PCLS) are an ex-vivo model that retain all lung resident cells and thus EVs derived from PCLS are likely to closely mimic EVs in-vivo. EVs were isolated from PCLS obtained from young (8-12 weeks) or aged (18 months) mice and characterised using nanoscale flow cytometry and atomic force microscopy. We employed the Acid Injury and Repair (AIR) model¹ to investigate the functional effects of EVs including their effects on apoptosis, progenitor cells, extracellular matrix and repair following injury in ex-vivo PCLS¹. RNA sequencing was conducted to analyse global differences between these EV populations.

RESULTS: EVs derived from young and aged PCLS (Young and Aged EVs) had similar size and concentration. Aged EVs were stiffer than Young EVs which was consistent with higher stiffness of aged PCLS compared to young PCLS. Young EVs decreased apoptosis when applied to injured lung tissue, from 66.4% to 35.7% in young PCLS and from 73.0% to 43.1% in aged PCLS, and Aged EVs showed similar effects. Treatment with Young EVs increased percentage of progenitor cells (proSP-C+ cells) in injured regions of PCLS and led to reduced type I collagen accumulation whereas Aged EVs did not. Differentially expressed miRNAs were identified between Young and Aged EVs.

DISCUSSION & CONCLUSIONS: These results indicate that EV tissue of origin has an impact on their repair capacity and that Young EVs have potential therapeutic value which should be investigated further.

ACKNOWLEDGEMENTS: Sally Yunsun Kim acknowledges the Wellcome Trust and National Heart and Lung Institute for the Institutional Strategic Support Fund Springboard Fellowship.

REFERENCES: ¹Kim SY and Mongey R et al. Biomaterials 2021, 267:120480.

Keywords: Disease models, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-054

Bioassembling Macro-Scale, Lumenized Airway Tubes via Multi-Organoid Patterning and Fusion

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INTRODUCTION: Engineered fusion of multicellular materials, such as spheroids and organoids, represents a biomimetic cell assembly process fundamental to the fields of biofabrication, tissue engineering and in vitro tissue modelling. Upscaled fusion of cystic epithelial organoids into shape-controllable, large lumenized tissues is challenging. This is because to produce large, functional epithelial tubes with an elongated lumen, such as the trachea, the fusion of organoid cysts requires not only the seamless surface integration between adjacent organoids but also the interconnection of their fluid-filled lumina.

METHODS: Starting with individual airway organoids derived from adult mouse tracheal epithelial cells, we established a robust organoid fusion platform, for the upscaled engineering of size-relevant epithelial organ tubes. Milli-wells were used to prescribe the initial tubular shapes of the organoid aggregates. The aggregates were then released to be cultured in a Matrigel-deflected, floating condition for the aggregates to undergo lumenisation.

RESULTS: Multi-organoid aggregates (MOA) in the floating culture exhibit a well-defined three-stage process of inter-organoid surface integration, luminal material clearance, and lumina connection. The organoid fusion efficiency under our condition exceeded ~90%. The observed shape stability of patterned MOAs is confirmed by theoretical modelling based on organoid morphology and the physical forces involved in organoid fusion. Immunofluorescent characterization shows that fused MOA tubes possess an unstratified epithelium consisting mainly of tracheal basal stem cells. The engineered airway MOA tubes, of a size similar to the mouse trachea (1–1.5 mm inner diameter), were perfusable. The partially-fused MOA can be used as a building block for further fusion to create a branching structure.

DISCUSSION & CONCLUSIONS: Our study suggests an alternative design pathway for epithelial organoid engineering, in which shape-patterning via molding is required only at the initial organoid assembly stage, while the prescribed geometry can be retained long-term in the floating culture. MOrPF engineered epithelial tubes are readily accessible for further coculture or matrix integration, and compatible with fluidic techniques to enable lumen access and fluid transport. We envisage that such macroscopic tubular epithelial building blocks could have broad implications in creating size-relevant, structurally complex, multi-tissue organ mimics.

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Keywords: Stem cells - general, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-055

Elastin-like hydrogels promote a physiological-like interaction with human primary lung fibroblasts

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INTRODUCTION: The field of lung regenerative medicine is still in its infancy, and it mostly relies on decellularized extracellular matrices, largely overlooking the potential role of biomaterials in improving regeneration. Among the different biomaterials, elastin-like recombinamers (ELRs) have shown to form hydrogels that promote the regeneration of different tissues, setting the grounds for their use in lung bioengineering applications [1]. Herein, as a first step, we show their interaction with primary human distal lung fibroblasts in terms of cytocompatibility and cell morphology.

METHODS: Methods

The ELRs used in this work were synthesized in the BIOFORGE Lab of the University of Valladolid (Spain). Primary human distal lung fibroblasts were isolated from healthy lungs rejected for transplantation, upon approval by the donor/next of kin and within specific ethical permits. The fibroblasts were expanded and cultured inside ELR hydrogels (3D) and on tissue culture plates/chamber slides (2D). For the 3D group, 10⁶ cells/mL were mixed with the ELR solutions prior to hydrogel formation, to allow the homogeneous embedding of the cells inside the scaffolds. In both cases, cytocompatibility was measured in terms of cell viability (LIVE/DEAD) and cell metabolic activity (resazurin reduction) at 0, 1, 3 and 7 days of culture. Moreover, actin (Phalloidin-iFluor 555) and nuclei (DAPI) were stained for the observation of cell morphology through confocal microscopy imaging after 7 days of culture.

RESULTS: Human primary distal lung fibroblasts were successfully seeded inside ELR hydrogels. They showed high cytocompatibility as per cell viability measurements, with similar values of living and dead cells compared to the 2D control. As regards metabolic activity, fibroblasts seeded in 3D showed a much slower increase in resazurin reduction than the ones seeded on 2D, maintaining stable levels after 1 day of culture. Finally, cells spread anisotropically in branches, contrasting with the isotropic expansion observed in the 2D group.

DISCUSSION & CONCLUSIONS: The excellent cytocompatibility shown by the ELR hydrogels provides preliminary data to support their use to promote lung regeneration. Furthermore, a lower metabolic activity increase correlates with a decrease in cell proliferation rate. This result, together with the different morphology found compared to the regular 2D culture, suggests that the cells may resemble more their homeostatic state where no high proliferation rates and isotropic expansion is found. Altogether, this work sets the ground for further investigations related to lung regeneration mediated by ELR hydrogels.

ACKNOWLEDGEMENTS: Fundación Ramón Areces (BEVP33S12276).

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Keywords: In vitro microenvironments, Biomaterials

OP-056

Engineered *in vitro* models to mimic the human alveolar barrier through biomaterials and advanced systems

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INTRODUCTION: Several chronic and acute lung diseases affect the properties and functions of the alveolar wall leading to serious and often fatal outcomes ¹. The development of effective therapeutic approaches is currently limited due to the lack of relevant preclinical models for their testing and validation ². To overcome ethical, experimental and technological limitations of the traditional *in vivo* and *in vitro* models, we exploit tissue engineering approaches to develop different biomimetic *in vitro* models to reproduce the two main portions of the alveolar wall: (i) a barrier model for the thin portion of the alveolar wall characterized by an alveolar epithelium and a pulmonary endothelium, separated by a thin basement membrane; (ii) a barrier model for the thick portion of the alveolar wall where an alveolar interstitial space is interposed between the alveolar epithelial and pulmonary endothelial basement membranes.

METHODS: A biomimetic thin barrier model was obtained by integrating an electrospun polycaprolactone-gelatin (PCL-Gel) membrane in ad-hoc modified transwell insert to mimic the alveolar basement membrane. Alveolar epithelial (A549) and lung endothelial (HULEC-5a) cells were cultured on the apical and basolateral side of the PCL-Gel membrane, respectively, under physiologic air-liquid interface (ALI) conditions. Moreover, a thick barrier model was developed by combining PCL-Gel membrane and fibroblasts (MRC-5)-laden collagen hydrogel and setting a tri-culture layering the alveolar epithelial cells, lung fibroblasts and lung microvascular endothelial cells. *In vitro* analyses were carried out to investigate the cellular morphology and barrier properties of the developed models. Model ability to emulate pathological conditions was also validated.

RESULTS: *In vitro* cell tests demonstrated that co-culture of A549 and HULEC-5a cells at ALI allowed to recreate a physiological-like environment promoting the expression of alveolar type I and type II epithelial cell markers as well as improving cell viability and barrier function. In addition, fluorescence images of the thick barrier model showed that cells maintained their typical shape after 7 days at ALI. Moreover, immunofluorescence staining of tight and adherent junctions demonstrated the formation of a tight barrier. The effect of lipopolysaccharides was evaluated showing the loss of barrier functions.

DISCUSSION & CONCLUSIONS: The obtained results suggest that the proposed systems could be used to reproduce the microenvironment of both healthy and pathological alveolar tissue.

ACKNOWLEDGEMENTS: This work was supported from the Regione Piemonte under the Digital tEchnology For Lung Cancer Treatment (DEFLECT) project.

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Keywords: Biomaterials, Microenvironment and niche engineering

OP-057

Novel organoid model of distal lung based on primary human cells to study mechanisms of lung diseases and responses to treatment

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INTRODUCTION: Despite impressive progress in development of human pulmonary organoid models, majority of these models are comprised predominantly of epithelial cells, with just a few reporting presence of supporting mesenchymal cells. These organoids do not yet recapitulate the complex structure and cellular interactions of the highly vascularized alveolar region. Therefore, more complex models utilizing the entirety of the lung architecture are required.

METHODS: We have devised a novel organoid model system that better recapitulates normal distal lung complexity. This model is based on the self-aggregation of primary human small airway epithelial cells (SAECs), pulmonary microvascular endothelia cells (HPMECs) and lung mesenchymal stromal cells (MSCs) in Matrigel.

RESULTS: These organoids develop alveoli-like budding structures with a lumen, which are positive for alveolar epithelial markers (Surfactant Protein C (SPC) and Aquaporin5, markers of Alveolar Type II (ATII) and Type I (ATI), respectively, and reach 200µm in diameter (this size is comparable to the size of human alveolus), remarkably, these organoids support the presence of endothelial cells up to 21 day. To test the ability of these model to recapitulate features of human disease, MSCs from healthy lungs were replaced by MSCs isolated from lungs of patients with COPD. Organoids seeded with MSCs derived from COPD lungs are characterised by non-symmetrical morphology and smaller size compared to organoids seeded with MSCs isolated from lungs of healthy donors and were unable to support endothelial cells, recapitulating loss of vasculature in emphysema. Both COPD lung MSCs and COPD organoids had lower levels of HGF10 secretion compared to their healthy lung counterparts which also is a characteristic feature of emphysema. Furthermore, organoids composed of COPD MSCs also demonstrate increased deposition of collagen compared to organoids composed of healthy MSCs. We also show that this model is amenable to model lung fibrosis and lung responses to treatment. Exposure of organoids to bleomycin (15 µg/ml for 72 hrs) resulted in increased accumulation of 1A1 collagen, which was reduced by the treatment with mesenchymal stromal cell derived extracellular vesicles.

DISCUSSION & CONCLUSIONS: Condensation of primary pulmonary cells provides a physiologically relevant distal lung organoid model that features endothelial cell presence. Lung MSCs are critical to support growth of endothelial cells and regular spatial organisation of the alveolar epithelial cells.

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Keywords: Extracellular vesicles, Disease models

OP-058

Human lung organoids predict response to carbon-based nanomaterials and model pulmonary fibrosis

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INTRODUCTION: Advances in in-vitro culture systems have stimulated an interest in more reliable testing approaches that predict nanomaterial toxicity risks and align with the 3Rs framework. Lung organoids are one example, and display functions consistent with their in-vivo counterparts. Organoids have been used in a variety of applications, but studies have yet to interrogate their suitability for nanomaterial testing. Here, we evaluate the potential of human lung organoids as tools for predicting pulmonary toxicity of carbon-based nanomaterials.

METHODS: Human embryonic stem cells were differentiated into multilineage lung organoids in 50 days, by adapting previously published protocols¹. Organoids were characterised using fluorescence microscopy, flow cytometry, live imaging and TEM. To exploit the airspace-like lumen of the organoids for cargo delivery, a novel microinjection exposure system that tailors the injection volume to each organoid was developed. The impact of small nanometric (50-950nm, sGO) and large micrometric (4-41µm, lGO) graphene oxide sheets, with multi-walled carbon nanotubes (1-20µm, MWCNT) on lung organoids after 1/7/28d exposure was then assessed. Endpoints included viability (AV/PI assay), histopathology (H&E staining), and cell-nanomaterial interaction (IF staining).

RESULTS: After 50-days of differentiation, the lung organoids exhibited the six major proximal (goblet, basal, club, ciliated) and distal (AECI/II) epithelial cell types of the adult lung. The organoids also contained functional cells, evidenced by active ciliary beating and deposition of surfactant/mucin in the organoid lumen. Consistent with published in-vivo data², both sGO and lGO exhibited a transient interaction with the alveolar epithelium (AECII/I), and did not induce any histoarchitectural changes indicative of fibrosis. Organoids did however produce 2.5-fold more mucins (MUC5AC/MUC1) in response to lGO than sGO exposure, suggesting that a thicker mucin barrier is required to prevent lGO-induced damage. In contrast, MWCNT caused detrimental effects on organoid architecture. Its persistent interaction with the alveolar epithelium led to a pro-fibrotic phenotype, where MWCNT caused AECII to undergo epithelial-mesenchymal transition (evidenced by an increase in α -SMA+/Vimentin+ mesenchymal cells and a decrease in EpCAM+ epithelial cells at d7).

DISCUSSION & CONCLUSIONS: Our human lung organoid exposure model has the potential to bridge the toxicological profiles between animals and humans. The model captures the complexity of cell-nanomaterial, cell-cell and cell-matrix interactions induced by exposure to carbon-based nanomaterials. With further development, this model may reduce the need for rodent inhalation studies in the assessment of lung toxicity.

ACKNOWLEDGEMENTS: This work is funded by the Graphene Flagship Core 3 Project, grant number 881603.

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[2] Rodrigues. 2020. Adv Sci. 7(12):1903200.

Keywords: Nanomaterials (inc graphene), Embryonic stem cells

OP-059

Aerosolization of endothelial cells for application in biohybrid oxygenators

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INTRODUCTION: Endothelialization of gas exchange membranes of extracorporeal membrane oxygenation (ECMO) systems is a promising approach towards improved hemocompatibility of ECMO devices. Current endothelialization techniques require large volumes of highly concentrated cell solution. This limitation could be overcome by cell aerosolization of endothelial cells on substrates with different topographies. Although general feasibility of cell aerosolization using laboratory prototypes was recently demonstrated by our group [1], a translation of the method towards a commercially available system is a first step towards clinical applicability. Thus, the aim of the current work is to analyze whether endothelial cells can withstand the stresses during aerosolization with a commercial system without changing their properties.

METHODS: A co-axial atomizer (Vivostat® system) was used for aerosolization of human umbilical vein endothelial cells with pipetted cells serving as control. The influence of the processing was evaluated via Live/Dead staining; proliferation rate of the cells after spraying was analyzed with XTT assay. Apoptosis and necrosis rate of endothelial cells were evaluated with CaspaseGlo and ToxiLight assays, respectively. Cells were cultivated under dynamic conditions on polydimethylsiloxane gas exchange membranes to evaluate if endothelial cells can withstand physiological wall shear stress (0.5 Pa). The cell confluency was evaluated with immunocytochemical (ICC) antibody staining against CD31 (PECAM-1) and von Willebrand factor (vWf).

RESULTS: The endothelial cell survival rate was higher than 90 % after aerosolization. Proliferation rate of the cells remained same as the pipetted cells for up to 5 days; no increase in apoptosis and necrosis levels was identified 24 hours after aerosolization. Both endothelial cells after aerosolization and the control formed a confluent cell layer and withstood wall shear stresses for 24 hours. ICC staining revealed a confluent endothelial cell layer with typical expression of CD31 and vWf as well as endothelial cell-typical cobblestone cell morphology.

DISCUSSION & CONCLUSIONS: The current study shows the feasibility of endothelial cell aerosolization on gas exchange membranes utilizing a commercially available aerosolization system. Aerosolization might reduce the amount of needed cells in comparison to conventional cell seeding methods.

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Keywords: Interfaces - biological, Bioreactors

OP-060

Development of a biomimetic scaffold for delivery of iPSC glial progenitors for spinal cord applications

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INTRODUCTION: Following spinal cord injury, a lesion cavity develops preventing axonal regrowth. Implants that bridge the cavity and encourage axonal growth while delivering trophic cells to restore lost tissue may have potential for repair. Therefore, by identifying neurotrophic extracellular-matrix proteins to incorporate into biomimetic hyaluronic acid scaffolds and by tuning the scaffold stiffness, we aimed to develop a novel scaffold platform capable of housing and supporting trophic iPSC-derived glial-progenitors for spinal cord repair.

METHODS: Spinal cord astrocytes and neurons were cultured on a range of matrix-proteins to identify a novel neurotrophic substrate. Following the incorporation of the neurotrophic substrate into 3D hyaluronic acid scaffolds of varying stiffnesses, scaffold physicochemical properties were characterized. Spinal cord astrocytes, neurons, dorsal root ganglia (DRG) and iPSC-derived astrocyte progenitors were cultured in scaffolds for up to 21 days and the effect of scaffold physicochemical properties was assessed. In addition, the impact of scaffold properties on the trophic capacity of iPSC-derived progenitors was assessed in vitro and ex vivo using neurons, DRG and spinal cord explants.

RESULTS: Collagen-IV (CIV) and fibronectin (FN) were observed to synergistically enhance neurite outgrowth by 203% while also promoting resting astrocyte phenotypes. Subsequently, hyaluronic acid scaffolds functionalized with CIV/FN were manufactured with different stiffnesses ranging from soft/biomimetic to stiff/supraphysiological (0.8-3kPa). Spinal cord astrocytes cultured in soft-CIV/FN functionalized scaffolds matching cord stiffness, elicited positive immunomodulatory responses and increased pro-reparative IL-10 release. Furthermore, soft scaffolds significantly enhanced neurite outgrowth from neurons and adult mouse DRG explant cultures compared to stiffer scaffolds. Soft-CIV/FN scaffolds also enhanced iPSC glial-progenitor neurotrophic and functional capacity while encouraging the growth of iPSC-derived spheroids that subsequently formed extensive neuronal/astrocytic cellular tracts within the scaffold. Conditioned media removed from soft-CIV/FN but not other, iPSC-loaded scaffolds and applied to growing neurons enhanced neurite outgrowth by 280%. Super-resolution imaging also demonstrated similar neurite enhancement (170%) in mouse primary neurons treated with media taken from soft-CIV/FN scaffolds. Media from stiffer or non-CIV/FN-containing scaffolds did not elicit a neurotrophic effect but instead increased pro-inflammatory IL-1 β release. Finally, when DRGs or spinal cord explants were cultured directly on CIV/FN iPSC-loaded scaffolds, only soft scaffolds enhanced cellular infiltration, astrocytic process extension and directed axonal outgrowth between DRG and iPSC-spheroids.

DISCUSSION & CONCLUSIONS: Overall, this work demonstrates the development of a novel, biomimetic scaffold-based platform and has significant therapeutic implications for spinal cord repair

applications

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Keywords: Biomaterials, Induced pluripotent stem cells

OP-061

Development of a bio-inspired *off-the-shelf* small diameter vascular graft

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INTRODUCTION: Cardiovascular disease is one of the major causes of death worldwide[1]. Autograft remains the gold standard but has limitations in supply and anatomical variability, while synthetic vascular grafts (SVGs) fail for vessels of diameter less than 6mm due to compliance mismatch[2]. Melt-electrowriting (MEW) is a recently developed fabrication method that allows for the layer-by-layer assembly, leading to a highly controlled micron-fibre architecture in 3D[4]. This can be tuned to mimic the collagen orientations of native vessel wall[3], providing mechanical and biological regenerative cues. Therefore, the aim of this project is to harness and build on MEW technology to develop a bioinspired multicomponent *off-the-shelf* small-diameter VG.

METHODS: A custom-made MEW[5] was developed to direct the deposition of PCL micron-scale-fibres in a bioinspired direction mimicking native vessel wall (angles 45°-26°-18°-11°). Infusion of fibrinogen into MEW scaffolds was optimized (pore size-porosity-degradation rate) for SMCs. Fibrinogen was functionalized with heparin to prevent clotting. The construct was wrapped in an electrospun PLCL sheath. Scanning electron microscopy was used to investigate morphological characteristics. Ring tensile test was used to investigate the mechanical properties. Biological evaluation was performed (cell proliferation/differentiation-ECM deposition/orientation) as well as hemocompatibility and endothelialisation. Bench tests were performed to access graft compliance to the ISO7198. Lastly, a rat abdominal aorta (AA) substitution was performed to test graft patency *in vivo*.

RESULTS: MEW produced grafts that mirror the orientation of collagen in native vessel. Different scaffold fibre angle recapitulated the typical anisotropic J-shape stress-strain curve of native tissue. MEW fibres acted as boundary conditions to drive cell and ECM orientation. Fibrinogen optimized infusion, structure, and degradation significantly enhance cell infiltration, ECM production, and endothelialisation, while ensuring the MEW framework could still influence ECM orientation. Heparin successfully implementation reduced platelet adhesion. PLCL allowed for a higher resistance to deformation and recovery, matching native compliance. The ISO-standards were satisfied with improved permeability, burst pressure and suture retention. The VG implanted in a rat's AA model proved good patency, reduced platelet and red blood cell adhesion.

DISCUSSION & CONCLUSIONS: We have successfully developed a bioinspired multicomponent *off-the-shelf* VG that is a promising alternative to limited current approaches and may represent a novel solution to address the unmet clinical need for small-diameter vascular substitutions.

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Keywords: Cardiovascular, 3D printing and bioprinting

OP-062

Exogenous lactate improves cardiac function of living myocardial slices

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INTRODUCTION: Lactate, an important metabolite during cardiogenesis and cardiac development, has been recently described as a potential modulator of the phenotype of cardiac cells *in-vitro*[1]. These findings support a novel use of lactate for endogenous heart regeneration strategies. Nevertheless, current evidence indicates that effectiveness of ongoing therapeutic approaches is dependent on the level of maturation of the targeted tissue. Hitherto, the regenerative capabilities of lactate in mature cardiac tissue have not been described. In this work we used living myocardial slices(LMS) as a model of mature cardiac tissue. LMS are 300 µm-thick slices of living myocardium with conserved physiological structure and function[2]. Human and rat adult LMS were treated with lactate to evaluate early cellular, molecular, and functionality changes related to myocardial reprogramming, cardiac structural rearrangements, and fibrosis.

METHODS: Left ventricular LMS were prepared from human (including healthy and failing hearts) and Sprague-Dawley rat hearts. To mimic heart damage in rat LMS, cryoinjury was produced using a cylindrical rod cooled down on dry ice(−78.5°C). LMS were maintained for 48h. under physiological mechanical load and electrical stimulation in circulating, oxygenated media at 37°C. In the treated group, L-Lactic acid was dissolved in the culture media. Viability, contractility measurements, and changes in gene and protein expression were assessed.

RESULTS: Functionally, lactate-treated(8mM) healthy human LMS displayed an increase in contractility (7.84±0.90 mN mm⁻² vs. 5.51±2.09 mN mm⁻² in the control group). Similarly, human failing LMS exhibited enhanced contractility (9.00±2.60 mN mm⁻² vs. 4.67±3.20 mN mm⁻²) and became stiffer. Expression of fibrotic, pluripotency transcription factors, and cardiomyocyte markers were detected.

Exposure of healthy rat LMS to higher concentrations of lactate(20 mM) did not affect LMS viability nor altered LMS contractile force. Again, lactate promoted rat LMS stiffening. In cryoinjured rat LMS, lactate drastically increased contractility and altered tissue remodeling in the region bordering the injury.

DISCUSSION & CONCLUSIONS: LMS provide a representative *in-vitro* platform for translational cardiovascular research. By using LMS, we were able to elucidate the effects of lactate in mature cardiac tissue. Exogenous lactate enhanced cardiac function in both human and injured rat LMS. Upregulation of transcription factors and cardiomyocyte markers may suggest an effect on partial cardiomyocyte reprogramming that would counteract the effects of tissue stiffening. Altogether, this study further supports the prospective use of lactate as a bioactive signal in new endogenous cardiac regeneration strategies.

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Keywords: Differentiation, In vitro microenvironments

OP-063

Seamless multi-channel peripheral nerve guidance conduits made from aligned conductive silk nanofibres

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INTRODUCTION: Peripheral nerves have the capacity to regenerate under certain conditions. It is possible to enhance this capacity by guiding axonal growth from the proximal to the distal end of the injury with specific biochemical or morphological cues. In this work, seamless nanofibrillar multi-channel silk fibroin conduits have been designed with tuned topography (nanofibrillar alignment) and biochemical composition (silk fibroin functionalisation). This is realised by overcoating dissolvable phosphate glass macrofibres fibres with silk nanofibers exploiting a nanofibre yarn module. The structures were rendered electrically conductive using recombinant reflectin obtained using an engineering biology approach.

METHODS: Silk fibroin cast films were dissolved in HFIP to obtain 6% w/v solutions, and then electrospun using a custom-built yarn module to overcoat sacrificial phosphate glass fibre (PGF) bundles. Multiple overcoated PGF bundles were then put together and further overcoated. The PGF bundles were then dissolved out in water to produce seamless multi-channel structures of silk nanofibers. The morphology of the multichannel conduits was elucidated and quantified using X-ray computer tomography.

RESULTS: Alignment of the nanofibrillar surfaces can be tuned by process variables with a minimum alignment of 10° against the PGF bundle axis. The same applies for lumen diameter, which can be modified by changing the bundle size. Multichannel conduits were obtained by two-step electrospinning and the dissolution of PGF and freeze-drying allowed for the production of hollow channel structures. The wet-state mechanical properties of silk nanofibrillar threads are very similar to those reported for peripheral nerve tissues (Elastic modulus between 10 and 15 kPa). Reflectin functionalisation of silk also improved silk proton conductivity by ~60%.

DISCUSSION & CONCLUSIONS: The process allows for the production of seamless multi-channel conduits with tailored diameters and topographical cues to the aligned nanofibers. The addition of recombinant reflectin to silk enhances proton conductivity, providing additional nerve guidance cues.

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Keywords: Fibre technology, Nervous system (brain-central-peripheral / disorders)

OP-064

Immune-instructive materials for tissue regeneration and healing

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INTRODUCTION: The immune system plays a key role in response to pathogens and foreign materials as well as orchestrating tissue homeostasis following injury. In addition to biochemical signals, immune cells also respond to physico-chemical cues in their microenvironment. This provides exciting opportunities for modulating immune cells' function by developing novel materials with immune-instructive niches. Wound healing is a complex biological process that involves a close crosstalk between immune and stromal cells. Particularly, macrophages and fibroblasts are known to play a central role in successful wound healing following injury, by driving regulated transition from acute inflammation to resolution of inflammation and healing. Dysregulation in any of these processes, such as in diabetic wounds, results in chronic inflammation and creation of non-healing wounds.

METHODS: Using a high-throughput screening strategy we screened a library of acrylates and methacrylates to identify homo and/or co-polymers that are able to modulate key functional properties of human macrophages and fibroblasts applying a mix of cellular and molecular readouts. We further developed functional coatings and microparticles using such polymers which were tested for their bio-instructive properties in vitro and in vivo.

RESULTS: A selection of polymers were shown to promote pro or anti-inflammatory phenotypes in macrophages, and were also able to modulate attachment and proliferation of fibroblasts in vitro. The ability of these polymers to modulate foreign body response (FBR) against subcutaneous implants, or their impact on chronic wound healing were investigated in relevant in vivo experimental models. These experiments revealed that through modulating macrophage and fibroblast phenotype these polymers can suppress FBR and promote tissue regeneration and wound healing without any additional exogenous mediators.

DISCUSSION & CONCLUSIONS: The ability to control biomaterials surface attributes provides a powerful tool for modulating the phenotype and function of immune and stromal cells with the aim of reducing detrimental pro-inflammatory responses and promoting beneficial healing responses. We argue that it is possible to use immune-instructive materials to harness the immune system to regulate inflammation and promote healing with potential applications in a host of other inflammatory conditions.

ACKNOWLEDGEMENTS: EPSRC EP/N006615/1

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3- Discovery of synergistic material-topography combinations to achieve immunomodulatory osteoinductive biomaterials using a novel in vitro screening METHOD: The ChemoTopoChip. Burroughs L. et al. 2021 Biomaterials; 271:120740.

Keywords: Immunity / immunomodulation / macrophage, Wound healing

OP-065

Development of a Bioartificial Glomerulus Filtration Barrier using a medium chain length Polyhydroxyalkanoate, a Sustainable and highly Biocompatible Polymer

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INTRODUCTION: The deterioration of the kidney glomerulus is due to either acute kidney injury (AKI) or chronic kidney disease (CKD), ultimately leading to end-stage renal failure (ESRF). [1] Current treatments are inadequate; haemodialysis does not address all other kidney functions and donor organs for transplantation are scarce. Research in kidney tissue engineering tries to seek for a treatment alternative. We focus on developing a bioartificial glomerular filtration barrier using a highly biocompatible bacterial polymer called Polyhydroxyalkanoates (PHAs) through Fused Deposition Modelling (FDM).

METHODS: Conditionally immortalised human podocytes (CIHP) and glomerular endothelial cells (ciGenC) were used to test the biocompatibility of the mcl-PHA. [2, 3] Polyhydroxyalkanoates production was carried out by fermentation using selected bacterial strains fed with specified carbon substrate. [4] The polymers produced were thoroughly characterised to understand their thermal, chemical, and physical properties. The PHA was subjected to 3D printing by FDM to engineer a kidney bioartificial filtration barrier.

RESULTS: Cytocompatibility test showed for the first time that PHAs were highly compatible with the glomerular cells, comparable to tissue culture plastic (TCP), making PHAs a great sustainable material for tissue engineering applications. PHAs produced a yield of around 40 to 60 g of polymer per 100 g dry cell weight (~40-60% dcw). The average molecular weight, Mw, was around 100,000 g/mol for copolymer PHA (mcl-PHA) and around 300,000 g/mol for homopolymer PHA (scl-PHA). Tensile testing confirmed the elastomeric nature of mcl-PHA and the rigidity of scl-PHA. The mcl-PHA has a low melting temperature (Tm) which is highly printable. The 3D printed design was a layered woodpile with a 200 to 300 µm range of strut width.

DISCUSSION & CONCLUSIONS: We have conclusively shown that PHA supports adherence and growth despite the well-known hydrophobicity. In future, the bioartificial filtration barrier developed using the combination of the PHAs, and two glomerular cell types will be assessed for its' ability to conduct haemofiltration. It is hoped that this strategy will eventually lead to the development of a complete bioartificial glomerular filtration barrier.

ACKNOWLEDGEMENTS: This is a collaborative work between the University of Sheffield and University of Bristol. The Government of Malaysia funds the PhD student under the Malaysia Department of Public Services.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

OP-066

3D-printed bioinspired bilayer composite scaffold embedding antibacterial nanofibrous microstructures for promoting diabetic wound healing

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INTRODUCTION: Advanced therapies based on hydrogel scaffolds for diabetic wound repair show remarkable achievements compared with conventional dressings. These were promoted by 3D printing for engineering hydrogels with controllable microporosity and structural features mimicking skin layers. Combining 3D-printed hydrogels with bioactive agents or micro/nano structures would further simulate the wound microenvironmental cues. Bestowing a 3D-printed hydrogel with the structural and functional characteristics of a nanofibrous component that recapitulates the native ECM and serves as drug delivery/wound healing accelerator, would greatly promote the healing process. The objective of the current study was to develop an innovative multifunctional 3D-printed bilayer wound healing scaffold made of a hydrogel composited with antibacterial nanofibrous microstructures embedding zinc oxide nanoparticles (nZnO) and DDAB-modified nZnO (D-nZnO) for diabetic wounds.

METHODS: nZnO and D-nZnO were prepared by chemical precipitation and embedded into polylactic acid nanofibrous microspheres (NF-MS) obtained by thermally-induced phase separation to generate antibacterial nZnO@NF-MS and D-nZnO@NF-MS hybrids. Formulated polymeric inks incorporating these hybrids or their components were characterized by SEM, XRD, FTIR, rheological and mechanical properties, water uptake and antibacterial activity. The composite inks were 3D-printed into bilayer 3D scaffolds composed of two layers with different porosities. These were examined for antibacterial activity and wound healing potentials using a diabetic wound rat model.

RESULTS: SEM revealed spherical nanofibrous microspheres embedding nZnO or D-nZnO and affirmed the porous structure of the composite 3D-printed scaffolds incorporating hybrid NF-MS. The composite scaffolds had an ultimate strength of 199.81 ± 5.33 KPa and compressive young's modulus of 213.85 ± 7.0 Pa. They showed pronounced antibacterial activity against *Staphylococcus aureus*. Wound healing findings obtained by morphological/morphometric analysis, histopathological examination, determination of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) content of regenerated tissue and microbiological monitoring indicated that the D-nZnO@NF-MS scaffold possessed the greatest wound healing effect with ~95% wound contraction accomplished in 14 days and favourable histological features.

DISCUSSION & CONCLUSIONS: The developed 3D printed wound healing scaffold merging the multiple therapeutic activities of nZnO, Zn²⁺, and DDAB as well as the physicochemical and structural properties of PLA NF-MS and those of the 3D-printed bilayer scaffold provide a versatile bioactive

material platform for diabetic wound healing and other biomedical applications.

ACKNOWLEDGEMENTS: We acknowledge funding from the Academic Thesis Research Fund (ATRF) committee, Faculty of Pharmacy, Alexandria University [grant number ATRF-420016].

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Keywords: 3D printing and bioprinting, Composite materials

OP-067

Rotational Internal Flow Layer Engineering (RIFLE): A new biofabrication technology for microscale stratified tissue assembly

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INTRODUCTION: Stratified tissue is common throughout human anatomy with notable examples in cardiac, dermal, intestinal, retinal and vascular tissue. Current bioprinting technologies are unable to attain the deposition resolution and cell densities necessary to form the microscale strata commonly observed in human tissue, features that are often the width of a cell or smaller. The limited use of native hydrogels, such as collagen, also hinders progression in the field. To assist in overcoming these challenges we have developed a unique biofabrication technology termed Rotational Internal Flow Layer Engineering (RIFLE), for assembling tuneable, multi-layered tissue-like structures.

METHODS: Small volumes of fluid hydrogels were added to the inner surface of a high-speed rotating tubular mould. By exploiting the fluid dynamic effect of rimming flow, hydrogels were transitioned into a stable thin layer. The application of a gelation process, such as cross-linker addition or temperature change gelled the hydrogel allowing the next layer to be applied. Progressively, a macroscale tube composed of discrete microscale strata was built up.

RESULTS: Initial process development using alginate hydrogel determined that layer thicknesses were a function of the mould rotational speed and hydrogel viscosity, permitting control over the microscale stratified dimensions. Cell encapsulation showed post-assembly viabilities of up to 97%, enabling the assembly of high-density (10^8 cells/ml) layers into heterogenous constructs featuring 7 independent cell populations.

RIFLE was then applied to the biofabrication of the vascular tunica media using the native tissue components; collagen and human Smooth Muscle Cells (hSMCs). Layered hSMC-collagen spacing experiments revealed microscale tissue layers at thicknesses down to 12.5µm. Such cell-width dimensions match those observed in human medial lamellar units, the microtissue layered element of the tunica media. Control of the post-assembly compaction process generated hoop stresses that aligned hSMCs in the circumferential direction, also mimicking native tissue. Finally, human induced pluripotent stem cells were differentiated into contractile hSMCs and assembled into multilayer collagen vascular vessels that exhibited a vasoactive response to contractile agonists.

DISCUSSION & CONCLUSIONS: The results show that RIFLE is capable of assembling microscale layer tissue units into macroscale tubular structures. The capacity to assemble cell-width layers in collagen, a hydrogel that is traditionally difficult to biofabricate, represents an important progression for stratified tissue assembly. These attributes alongside the simplicity and low-cost of the system will, we hope, enable researchers to economically create a range of representative stratified tissue. Future work will seek to determine if more complex macroscale geometry can be formed using RIFLE.

Keywords: Enabling technologies, Microenvironment and niche engineering

OP-068

Advanced next-gen microcarriers for effective and efficient cultivation of induced pluripotent Stem Cells (iPSCs)

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INTRODUCTION: Induced pluripotent stem cells (iPSCs) are of great promise for a wide variety of applications, being used for development of cell therapies, screening of new drugs, and modeling of pathologies¹. However, challenges in mass expansion of iPSCs remain a key hurdle to clinical translation². Although microcarriers could potentially address this challenge, they often are very expensive, ineffective (cell adhesion of <80%), and inefficient (loss of pluripotency), which is in part caused by the unnatural stiff nature of most microcarriers. To this end, we designed an innovative soft microcarrier for efficient and effective bioreactor culture of iPSCs. These next-gen, soft, cell-adhesive microcarriers were produced in monodisperse ultra-high throughput manner by combining soft biomaterials with In-air microfluidics (IAMF) ³, which is a technology that was recently invented in our lab.

METHODS: To allow for the production of microcarriers with a desirable 3D shape, mechano-instructive properties, and bio-adhesive surfaces, we first prepared a blended solution (1:1) of alginate (80-120 cP) and PEG-diamine (Mn ~1,500) for physical and subsequent chemical crosslinking. To generate microgels, a piezo-actuated microjet composed of 1% (w/v) blend was collided with a continuous microjet of 0.01 M CaCl₂ and 10% (v/v) ethanol (EtOH). The surface biofunctionalization was performed from the electrostatic interaction between microgels and polyelectrolytes functionalized with RGD-peptide moieties

RESULTS: IAMF technology proved to be a viable technology for the reliable ultra-high throughput generation of monodisperse soft microcarriers with excellent surface biomodification capacity. After three days of culture, numerous cells had adhered to the surface of IAMFs microcarriers. Nine days post-seeding, many of the IAMFs microcarriers were linked together by cells, and numerous tridimensional structures were created by interaction between adherent cells. Enzyme-free cell detachment of cells from the microcarriers using bio-ionic liquids was demonstrated allowing for superior, efficient, and more cell-friendly cell harvesting as compared to traditional methods.

DISCUSSION & CONCLUSIONS: Here, we demonstrate IAMFs as a promising technology for ultra-high throughput production of monodisperse soft monodisperse microcarriers with adjustable mechanical properties in a manner that allows accurate control over the chemical structure of surface components for efficient cell expansion.

ACKNOWLEDGEMENTS: Financial support was received from the European Research Council (ERC Starting Grant, #759425) and EFRO - IPSC'S (#309-23330).

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Keywords: Biomaterials, Induced pluripotent stem cells

OP-069

Biofabrication of bioceramic scaffolds with different hollow strut geometries

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INTRODUCTION: Biofabrication of hollow-strut bioceramic scaffolds is an attractive strategy for enhanced performance in patient-tailored bone tissue engineering (BTE). The channels in such scaffolds offer nutrient and cell transport pathways and facilitate effective osseointegration and vascularization [1].

However, most studies into hollow-strut bioceramic scaffolds have involved only tubular structures with hollow cylinder geometries. Therefore, a systematic study of different strut geometries and their fabrication is important towards the improved design and fabrication of such bioceramic scaffolds. A logical approach that is worthy of exploration is the fabrication of hollow-strut scaffolds with varying outer (strut) and inner (channel) geometries.

METHODS: In this work, hollow-strut bioceramics scaffolds were fabricated by robocasting of precipitation-derived copper doped diopside. We describe the design and production of novel nozzles with various geometries via vat photopolymerization 3D printing. The customized nozzle enables the fabrication of scaffolds with hollow struts of diverse cross-sectional geometries. Microstructure and rheological characterization were studied. In addition, the mechanical properties of the scaffolds were investigated both by experiments and finite element analysis (FEA), and the potential applications of hollow struts for bone repair were investigated.

RESULTS: Copper-doped diopside $\text{CaMg}_{1-x}\text{Cu}_x\text{Si}_2\text{O}_6$ bioceramic scaffolds [2] with hollow members of varied outer and inner geometries (1. Outer triangle inner circle; 2. Outer quadrangle inner circle; 3. Outer pentagon inner circle; 4. Outer hexagon inner circle; 5. Outer circle inner triangle; 6. Outer circle inner quadrangle; 7. Outer circle inner pentagon; 8. Outer circle inner hexagon; 9. Outer circle inner circle; 10. Outer circle inner solid, ten scaffolds in total) were fabricated by robocasting through designed extrusion nozzles.

An optimized paste with favorable rheological behaviors was developed by employing alginate and Pluronic F-127 as additives. Mechanical testing and FEA reveal that hollow-strut bioceramic scaffolds with square cross-sections showed the best performances, exceeding those with round cross-sections or others. This arises due to lower stress concentration at scaffold intersections. The hollow structure of these bioceramic scaffolds significantly improved cell attachment and proliferation. Moreover, hollow members can be infiltrated with a second phase, in a subsequent step or during printing, to produce high-performance biomimetic composites and self-healing materials.

DISCUSSION & CONCLUSIONS: This work provides an easily controlled method for 3D printing of hollow-strut bioceramic scaffolds with various cross-sections, which is a reference for the development of diversity and mechanical enhancement of printed objects in extrusion-based 3D printing.

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Keywords: Biofabrication, Ceramics

OP-070

Biofabrication of microtissue-derived constructs for articular cartilage repair

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INTRODUCTION: Engineering functional and phenotypically stable articular cartilage is considered one of the greatest challenges in tissue engineering. Modular tissue engineering strategies that use cellular aggregates, microtissues or organoids as building-blocks can potentially be used to fabricate complex hierarchical tissues at scale. The aim of this work was to (i) assess the capacity of cartilage microtissues at different levels of maturation to form scaled-up cartilage grafts in the presence or absence of a temporary supporting hydrogel, and (ii) assess if extrusion based bioprinting of such cartilage microtissues could be used for the biofabrication of functional cartilage grafts.

METHODS: Mesenchymal stem cell (MSC) derived microtissues were fabricated as described previously (Nulty, Burdis and Kelly, 2021 and Burdis et al., 2022). These microtissues were harvested after 2 or 4 days of maturation, manually seeded in agarose wells with or without a supporting oxidized alginate and maintained in chondrogenic culture for 6 weeks in either static or dynamic culture conditions. As proof of concept, the microtissues were 3D bioprinted using an oxidized alginate-gelatin-based bioink (Barceló et al. 2022).

RESULTS: Microtissues that were matured independently for 2 days prior to seeding in oxidised alginate hydrogels generated higher amounts of glycosaminoglycans (GAGs) compared those matured for 4 days. Histological analysis revealed intense staining for GAGs and negative staining for calcium deposits. In contrast, microtissues that were not encapsulated into supporting oxidized alginate stained positive for calcium deposits. These differences in tissue development due to incorporation of a supporting temporary hydrogel were also observed in dynamic culture conditions, where encapsulation in oxidised alginate support a 2 fold increase in sGAG deposition. Furthermore, the microtissues (day 2 maturation level) were 3D bioprinted using an oxidised alginate based bioink. It was observed that the microtissues remained viable after the bioprinting process, were able to fuse after 48h, and generated a cartilage tissue that was rich in GAGs and negative for calcium deposits after 6 weeks in culture.

DISCUSSION & CONCLUSIONS: The key conclusions from this study are that: (1) less mature cartilage microtissues (day 2 maturation level) generate superior scaled-up cartilage grafts; (2) dynamic culture has a positive effect on the chondrogenic capacity of the constructs when compared to static conditions; (3) microtissues can be successfully used as in extrusion based bioprinting to engineer scaled-up cartilage constructs. This work supports the continued use of cartilage microtissues as biological building blocks in diverse biofabrication platforms.

Keywords: Biofabrication, 3D printing and bioprinting

OP-071

Scalable Microfluidic Biofabrication of a Modular, Immunoprotected and Microporous Mini-Pancreas for Type 1 Diabetes Treatment

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INTRODUCTION: Type 1 diabetes is an autoimmune disease in which the insulin producing pancreatic β -cells are destroyed by the body, which leads to unregulated blood glucose levels. Current treatments are either temporary/invasive (insulin injections), lack availability (pancreas transplants), or are sensitive to immunological rejection, as well as poor vascular integration (transplantation of pancreatic islands).^{1,2,3} To improve upon these drawbacks, we aim to engineer immunoprotected mini-pancreases composed of β -cells within microgels. Moreover, enzymatic annealing of the microgels allows for the engineering of macro-sized living matter with an open, highly dense, and interconnected internal pore network resembling the native tissues capillary network, which could be achieved in a highly scalable manner. This yielded an immunoprotective and retrievable miniature pancreas with improved diffusion properties as compared to traditional bulk hydrogel implants.

METHODS: Immunoprotective microgels were generated using a flow focusing microfluidic droplet generator in which tyramine conjugated polyethyleneglycol (PEG-TA) containing cells was emulsified using n-hexadecane with span-80. After emulsification, PEG-TA was crosslinked downstream in an outside-in manner using cytocompatible levels of hydrogen peroxide and horseradish peroxidase. This generated core-shell microgels in a robust, reproducible, and high throughput (~120 Hz) manner. Selective permeability of microgels was tested using small fluorescent molecules. Microgels were annealed into microporous annealed particle networks (MAPs) by densely packing microgels into a mold and crosslinking the residual tyramine groups using ruthenium, sodium persulfate, and visible light, resulting in a highly permeable microporous construct.

RESULTS: Functional microtissues were produced by encapsulating cells of the insulin producing β -cell line MIN6 within the hollow core of the microgel at a high level of viability. Microencapsulated cells were able to rapidly aggregate and form glucose responsive cellular spheroids within the hollow microgels, which remained functional for at least 30 days. Spheroid functionality was demonstrated using immunofluorescent staining of glucagon and insulin. Finally, these immunoprotected microtissues can be crosslinked into a MAP using the aforementioned visible light crosslinking system while maintaining high viability.

DISCUSSION & CONCLUSIONS: Here, we present a bottom-up bioengineered, modular mini pancreas through the covalent bonding of encapsulated, immunoprotected microtissues. Currently, we are testing the in vitro and in vivo functionality of the mini pancreas as a treatment for type 1 diabetes.

ACKNOWLEDGEMENTS: Financial support was received from the European Research Council (ERC, Starting Grant, #759425).

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Keywords: Diabetic healing, Vascular systems / vascularisation and heart

OP-072

Bioprinting of human dermal microtissues precursors as building blocks for an endogenous *in vitro* dermis equivalent

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INTRODUCTION: State-of-the-art three-dimensional *in vitro* human skin equivalent models are made of cells embedded in exogenous matrices (e.g., de-cellularized/de-epidermized dermis, collagen-based hydrogels and synthetic polymers)(1). However, these approaches fail to effectively reproduce the native dermis regulatory functions, since the biological communications are limited to cell/cell and cell/exogenous materials. Indeed, skin equivalents produced by fibroblast-derived dermal matrix, in which cells deposit their own ECM, represent a more faithful *in vitro* replica of native and functional tissues. Herein, we biofabricate an endogenous human dermis equivalent, using human dermal microtissues precursors (HD- μ TPs) as bioprinting building blocks.

METHODS: HD- μ TPs were obtained via dynamic seeding of Human Lung fibroblasts (HLF) inside biodegradable gelatin microcarriers (2), by means of a spinner-flask bioreactor. After 9 days, HD- μ TPs were suspended (50%v/v) in a solution of Pluronic-F127 (30%w/v) (P30- HD- μ TPs) and integrated into the dispensing syringe of Rokit INVIVO-4D6 bioprinter, as bioink. The bioprinting process was set-up to extrude multiple shapes (line, circle, square) inside a Pluronic-F127 (40%w/v) (P40) support bath, able to retain the bioink during and after the bioprinting process, until the HD- μ TPs formed a continuum of connective tissue. After 7 days, Pluronic was thermally removed, and the endogenous construct let mature to form a dermis equivalent. HLF behaviour and ECM morphology were examined over 28 days of culture on cross-section via immunohistochemistry (H&E, Masson Trichrome, Picrosirius Red, α -SMA, hyaluronic acid, elastin and SHG for collagen). The bioink and support bath rheological properties were assessed to select the suitable formulations prior bioprinting.

RESULTS: Rheological analysis confirmed: P30-HD- μ TPs bioink shear-thinning behaviour and temperature-dependent viscosity (gel at $T > 30^{\circ}\text{C}$), leading to HD- μ TPs homogenous dispersion and bioink printability; P40 support bath shear-recovery property, allowing the bioink retainment over 7 days, during HD- μ TPs fusion, to aid the connective tissue formation. The bioprinted macro tissues retained the initial shape and the collagen was uniformly distributed, by SHG reconstruction; α -SMA was present in very low amount, indicating the maintenance of fibroblasts phenotype; hyaluronic acid and elastin were found within the bioprinted tissues.

DISCUSSION & CONCLUSIONS: Our novel two-steps approach, based on the use of HLF-seeded microcarrier as bioprinting building blocks, allowed to re-create *in vitro* the native 3D stromal context, via the ECM/ECM interaction among HD- μ TPs leading to their fusion. By coupling such tissues with different epitheliums, it would possible to obtain a library of human organs, in which the corresponding connective part recapitulates the ECM turnover process.

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Keywords: 3D printing and bioprinting, *In vitro* microenvironments

OP-073

Biofabrication of biomimetic meniscal grafts via the controlled deposition of meniscus progenitor cells within wedged-shaped melt electrowritten scaffolds

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INTRODUCTION: Here, we report a novel method to engineer organized soft tissues, with a collagen architecture and mechanical properties similar to the native meniscus. By using fibronectin selected meniscus cells, we could engineer highly organized fibrocartilage tissue. The overall goal of this study is to assess whether these organized structures can be scaled up to obtained wedged-shaped constructs mimicking the native meniscus.

METHODS: MEW was employed to fabricate polycaprolactone (PCL) scaffolds with a defined geometric architecture including the complex wedge-shaped macro-architecture of the human meniscus. Meniscus progenitor cells were selected by using a fibronectin adhesion assay after tissue digestion. A custom alginate based bioink was developed and inkjet bioprinting was used for the dispensing of cell-laden bioinks into scaffolds.

RESULTS: Bioprinted cells deposited within the defined boundaries provided by the PCL fibres generate a fibrocartilage-like tissue, where neo-matrix organization is determined by the architecture of the scaffold. When meniscus progenitor cells were incorporated into MEW fibrous scaffolds with defined architecture, we could obtain anisotropic collagen networks resembling those of the native meniscus. The mechanical strength of the engineered tissues was similar to the native tissue in compression. Furthermore, meniscus derived cells produced a more meniscus-specific extracellular matrix compared to bone marrow derived mesenchymal stem cells. We next fabricated a larger scaffold replicating the wedged-shaped profile of the native meniscus. The pore architecture of these large scaffolds remained open even at the highest sections, enabling the bioprinting of cells and facilitating tissue growth throughout the entire scaled-up construct. By using populations derived from the inner or outer zone of the meniscus in relevant areas of the scaffold, we could obtain regional distinct tissues that recapitulated key features of the native meniscus.

DISCUSSION & CONCLUSIONS: In the present work, we have successfully developed a biofabrication approach that allows precise control over the orientation of the deposited collagen tissue. By using MEW as a fabrication technology, we could better mimic not only the collagen network architecture of the native tissue, but also engineer tissues with similar anisotropic mechanical behaviour. We have also succeeded in the fabrication of large volume MEW scaffolds of up to 5 mm height with well-defined micro-fibrous and macro-architectural features. This work demonstrates the potential of integrating MEW and bioprinting to engineer structurally organised soft tissues.

Keywords: 3D printing and bioprinting, Biofabrication

OP-074

3D PRINTING OF PCL-MgFe₂O₄ COMPOSITE SCAFFOLDS FOR ENHANCED BONE REGENERATION AND HYPERTHERMIA TREATMENT

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INTRODUCTION: The two critical needs of cancer management after tumor resection: (i) restoration and regeneration of the resected tissue and (ii) prevention of tumor recurrence by killing the residual tumor cells post-surgery. The current state-of-art cancer management addresses these two needs separately, the first one by autologous grafting and the second need by chemotherapy / radiotherapy. In our research, we aim to fabricate a dual-purpose biocompatible composite scaffold made of Polycaprolactone (PCL) and magnesium iron oxide (MgFe₂O₄) nanoparticles using a melt extrusion 3D printing process, which can be used in hyperthermia treatment via the application of an external magnetic field.

METHODS: The scaffolds were fabricated by dissolving PCL pellets in acetic acid through ultrasonication (40% w/v) for 5 hours. Magnetic nanoparticles (MNPs) were then added to the PCL solution of varying concentrations (2.5% - 10% w/v) and ultrasonicated. Melt extrusion was used to print the scaffolds (0.41 mm diameter needle, 0.06 MPa pressure and print bed temperature of 1°C).

RESULTS: The printing of scaffolds needs to take place soon after ultrasonication, since the MNPs agglomerate after a while. The EDX analysis of the PCL-MNP scaffolds showed a good distribution of MNPs throughout the scaffold. For all the different concentration of scaffolds, statistically significant cell growth over a 7-day period was observed (Alamar Blue assay). The compression tests indicated 10% MNP concentration scaffold having the highest modulus while the pure PCL scaffold showed the least modulus before yielding.

DISCUSSION & CONCLUSIONS: Ultrasonication is the key to achieving a fairly uniform distribution of MNPs. Time taken between the solution preparation and printing is key as longer time will result in separation into layers. Successfully-fabricated PCL-MNP scaffolds can be used for hyperthermia treatment as the application of an external magnetic field on MNPs in the sub 30 nm range [1] helps in the creation of a magnetic field driven microenvironment which enhances cell activity and calcium production leading to increased bone tissue regeneration [2-3]. This is partly the reason why the AB assay shows increased cell proliferation even for scaffolds with higher concentration of MNPs.

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Keywords: Biofabrication, Nanomaterials (inc graphene)

OP-075

A multimaterial and multifunctional patch for the repair of spinal cord injury

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INTRODUCTION: Spinal cord injury causes motor, sensory, and autonomic dysfunction. Conventional treatments for this disease are ineffective for functional recovery, so current research is interest in cell-based therapies when paired with a three-dimensional platform. Considering that, our patch explores the combination of the excellent biological properties of decellularized methacrylate adipose-derived extracellular matrix (adECM-MA) hydrogel with the enhanced neuronal differentiation powered by graphene-like multisheets (GLMS). To complement this, a melt-electrowriting polycaprolactone (MEW-PCL) grid to support the stability of the ultrasoft adECM-MA hydrogel was incorporated.

METHODS: Using a new methacrylation protocol for the adECM (provided by Tecnalía [1]) that could uphold a stable pH throughout the entire procedure, the adECM-MA hydrogels could be prepared via photo-polymerization with different concentrations of adECM-MA (0.25%, 0.5%, and 1% w/v), to select the most appropriate concentration for the soft spinal cord tissue. The hydrogels were then combined with 0, 5, 10, and 15% (w/w) of GLMS (provided by Graphenest SA). The MEW-PCL grid was printed with optimised melt-electrowriting in a branched microfibrillar structure to mimic the neuronal network. Neural stem cells (NSCs) were encapsulated, and their responses regarding viability and neuronal differentiation were assessed.

RESULTS: Compressive tests revealed a Young modulus of 5.57 ± 2.95 kPa for 0.25% adECM-MA, 7.58 ± 2.20 kPa for 0.5% adECM-MA, and 16.12 ± 4.11 kPa for 1%, wherein the elastic modulus was directly dependent on the hydrogel concentration, thereby demonstrating the tunability of mechanical properties of these hydrogels over a large range of Young's moduli. A soft, yet easily handled construct was obtained with 0.25% adECM-MA combined with the MEW-PCL grid, that showed to support the viability of the encapsulated NSCs. Furthermore, MEW-PCL grid revealed enhanced neuronal differentiation with the presence of long neuronal processes. When adding GLMS to the hydrogel, 5% and 10% showed better response in terms of neuronal differentiation with neurites up to 600 μm .

DISCUSSION & CONCLUSIONS: By implementing a 3D-printed PCL branched-microgrid within a hydrogel of a methacrylated decellularized extracellular matrix and GLMS, a multimaterial and multifunctional patch for the neuronal tissue of the spinal cord was conceptualised. The topographical and chemical signals of the grid and GLMS, respectively, direct neuronal differentiation of neural stem cells in this patch.

ACKNOWLEDGEMENTS: This work was supported by H2020-FETOPEN-2018-2020, NeuroStimSpinal No. 829060).

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Keywords: 3D printing and bioprinting, Nervous system (brain-central-peripheral / disorders)

OP-076

Encapsulated vaterite-calcite CaCO_3 particles loaded with bioactive cations with sustained release promoting osteogenesis and angiogenesis in vivo

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INTRODUCTION: Bioactive cations, including calcium, copper and magnesium, have shown the potential to become the alternative to protein growth factor-based therapeutics for bone healing. Ion substitutions are less costly, more stable, and more effective at low concentrations. Although they have been shown to be effective in providing bone grafts with more biological functions, the precise control of ion release kinetics is still a challenge. Moreover, the synergistic effect of three or more metal ions on bone regeneration has rarely been studied. In this study, vaterite-calcite CaCO_3 particles were loaded with copper (Cu^{2+}) and magnesium (Mg^{2+}). The polyelectrolyte multilayer (PEM) was deposited on CaCuMg-CO_3 particles via layer-by-layer technique to further improve the stability and biocompatibility of the particles and to enable controlled release of multiple metal ions.

METHODS: • CaMgCu-CO_3 were prepared by co-precipitation, further encapsulated with PEM and collagen fiber.

- Characterization: QCM, zeta-potential, SEM and flu-microscope for the coating process and morphological characterization. The release kinetics of multi-metal ions were recorded by ICP-OES over 60 days.
- In vitro study with preosteoblast-like cell lines: biocompatibility assay, alkaline phosphatase activity test, relative gene expressions of angiogenic markers and mineralization staining were performed.
- In vivo study with animal bone defect models: histopathological and immunopathological analyses.

RESULTS: In this study, CaCO_3 -PEM-Col microcapsules showed improved stability and controlled release of Ca^{2+} , Cu^{2+} and Mg^{2+} for over 60 days. The results of in vitro studies showed that CaCuMg-PEM-Col provided overall the optimal effects on osteogenesis and angiogenesis compared with CaCu-PEM-Col , CaMg-PEM-Col , Ca-PEM-Col . Interestingly, in the rabbit bone healing process, the CaMg-PEM-Col showed the significantly improved bone tissue regeneration and vascularization compared to other groups and controls. This may be due to the different ion release behaviour of this delivery system and different synergistic effects on the bone healing process in vivo.

DISCUSSION & CONCLUSIONS: This study indicates that such a functional and controllable delivery system of multiple bioactive ions might be a safer, simpler and more efficient alternative to protein growth factor-based therapeutics for bone regeneration. It also provides an effective method to improve the osteoinductivity of bone substitute materials.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

OP-077

Exploring Angiogenic Properties of Bioactive Glasses From the SiO₂-CaO-Na₂O System

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INTRODUCTION: Angiogenesis is an essential process for successful bone regeneration and repair. This process is particularly indispensable in the repair of critical-sized bone defects, which remain challenging in the clinical setting as a lack of vascular network can lead to insufficient nutrient and oxygen supply with subsequent graft failure. Bioactive glasses are a group of ceramic biomaterials finding numerous applications in clinics due to their excellent biocompatibility and bioactivity. In this study, we investigated the angiogenic properties of a novel type of bioactive glass. Preliminary in vivo tests using a zebrafish model suggested that some of these glasses possess angiogenic properties.

METHODS: Five bioactive glasses from the SiO₂-CaO-Na₂O system were previously synthesized from calcite minerals and silica sands extracted from natural deposits in Cuba.

To characterize the atomic and molecular structure of the materials, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction spectroscopy (XRD) were performed. The biological properties of five bioactive glasses were tested through a direct and indirect culture with human umbilical vein endothelial cells (HUVECs). Cell proliferation and metabolic activity were investigated on days 1, 3, 7, and 14 through a direct culture with bioactive glass particles at concentrations of 100, 200, 300, 400, and 1000 µg/mL. Further investigations were continued with the best-performing formulations. To this end, tubule-like structure formation and cell migration assays were performed. Finally, angiogenic properties were further confirmed in a chick chorioallantoic membrane (CAM) model.

RESULTS: The amorphous character of the tested bioactive glasses was confirmed by the XRD analysis. FTIR spectra revealed the composition of the bioglasses, while XRD patterns suggested the presence of nanocrystalline domains. In vitro experiments showed an overall increase in proliferation over the period of 14 days. Toxic concentrations identified with metabolic assay were excluded from further analysis. Three bioglass formulations at concentrations of 200 and 400 µg/mL were chosen for further tests. Culture with bioglass conditioned medium showed altered cell arrangements in comparison to the untreated control demonstrating tubule-like structure formation. Furthermore, bioactive glasses tested in a CAM model influenced the number, distribution, and branching of the blood vessels.

DISCUSSION & CONCLUSIONS: Out of the five initial bioglass formulations, three were identified as the most potent regarding angiogenesis. Overall, our results demonstrated a correlation between different material compositions with the angiogenic properties of these materials. Future work will focus on an in-depth investigation of the osteogenic properties of bioactive glasses.

Keywords: Ceramics,

OP-078

A decellularized dermal matrix scaffold suitable for cardiac tissue engineering is remodeled by resident human cardiac progenitor cells in vitro

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INTRODUCTION: The role played by ECM in myocardium integrity and homeostasis has been emphasized by cardiac tissue engineering (CTE) and decellularized ECM has rapidly emerged as an attractive biomaterial. We have recently provided evidence in support of the decellularized human skin (d-HuSk) as a promising biomaterial for CTE (1). Since in vivo ECM is in dynamic equilibrium with cells, the scope of the present study is to evaluate in vitro whether d-HuSk is remodeled by resident human cardiac progenitor cells (hCPCs).

METHODS: To this purpose, human skin from patients undergoing abdominoplasty was decellularized and cryosectioned to prepare three-dimensional scaffolds, while hCPCs were isolated from explanted human hearts, seeded on d-HuSk scaffolds, and cultured for two weeks. Afterwards, hCPCs on d-HuSk were induced to myogenic differentiation and cultured for two additional weeks.

RESULTS: The absence of nuclei in H&E staining and a DNA content of 9.138 ± 1.009 ng/mg of tissue confirmed the decellularization efficiency. Real-time PCR analyses revealed a significant up-regulation in transcription of differentiating and mature cardiomyocyte genes, along with retained transcription of mesenchymal, endothelial, and smooth muscle cell genes in hCPCs induced to differentiate. SEM analysis showed a better organized network of elastic fibers in hCPC-d-HuSk bioconstructs than in acellular d-HuSk, which resulted richer in collagen thicker fibers. Furthermore, quantitative analyses of scaffold composition revealed in hCPC-d-HuSk the significant increase of GAGs and decrease of collagen and elastin, along with a remarkable higher fluorescence mean intensity for fibronectin and tenascin.

DISCUSSION & CONCLUSIONS: The results showed confirm the hypothesis that d-HuSk is actively restructured and remodeled by hCPCs, thus providing additional data supporting that d-HuSk could be an effective temporary platform for CTE.

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Keywords: Biomaterials, Cardiovascular

OP-079

Synthesis and characterization of novel selenated hydrogels for tissue engineering and 3D bioprinting

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INTRODUCTION: The development of new materials that allow three-dimensional cell culture is necessary to achieve greater similarity to natural tissues. Hydrogels are one of the most common tissue engineering scaffolds due to their ability to support a 3D structure, to provide mechanical support for cells, and to simulate the native extracellular matrix. Also, they can be chemically functionalized to display additional features. Selenium has been shown to have relevant actions due to its antioxidant and anticarcinogenic properties. Recent studies have confirmed a correlation between the decrease of the level of blood plasma Se in patients with acute coronary syndrome and myocardial infarction. Thus, the use of selenated hydrogels could be very beneficial in the field of cardiac tissue engineering. The aim of the present work is to incorporate selenium to the polymeric backbone of hydrogels, and to generate a final system capable of biorthogonal photopolymerization. **METHODS:** Chemical synthesis. Different strategies and crosslinking strategies have also been tested to form the hydrogels from the functionalized gelatin. These are based on the Diels-Alder and Michael reactions, stimulated by photopolymerization with visible light.

Hydrogel characterization. These have been NMR, atomic absorption, ninhydrin assays, rheometry for mechanical characterization, and photopolymerization tests.

Cell culture viability assays. The cell lines used were HELAs, mesenchymal stem cells and cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs). The viability assays used were the AlamarBlue and LIVE/DEAD tests. hiPSC-cardiomyocytes were obtained in-house by differentiation through small molecule-based biphasic modulation of the Wnt pathway, followed by lactate-based metabolic selection.

RESULTS: Characterization of the new molecules not only confirms the acquisition of the desired functionality, but also gives insight to their structure. The synthesized selenated gelatin derivatives are capable of photopolymerizing to form hydrogels (Figure 1), even without concurrence of photoreactive groups. Rheometry tests show that it is possible to vary the mechanical properties of hydrogels by changing the percentage of gelatin, the concentration of photoinitiators or the time of exposure to light. Cell viability has been found to be similar to other biomaterials such as GelMA. We have also been able to verify that it is a material suitable for 3D printing.

DISCUSSION & CONCLUSIONS: New Se-containing gelatin derivatives have been synthesized, which can biorthogonally photo-polymerize to form hydrogels. Some of the new hydrogels do not only form a 3D environment for cell culture, but also provide biological function by containing Se.

Keywords: Biomaterials, Cardiovascular

OP-080

Recombinant spider silk for cardiovascular applications: Biodegradable, drug eluting, and endothelial cell-specific materials

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INTRODUCTION: Engineered recombinant spider silk possess outstanding mechanical properties and biocompatibility. Furthermore, it significantly reduces foreign body reactions of coated implants. In this work, we illustrated the potential of recombinant spider silk in cardiovascular applications. Specifically, we recombinantly expressed spider silk proteins modified with the endothelial cell-specific REDV peptide. We then illustrated that the materials exhibit specific attachment of endothelial cells, excellent haemocompatibility, enzymatic degradability, and can be used for the delivery of clinically relevant drugs.

METHODS: Cell selectivity and proliferation: Endothelial cell selectivity was observed by 4 hr attachment tests of different human cell types including human umbilical cord endothelial cells (HUVEC) on protein films. HUVEC were cultivated for 7 days various silk variants to observe the proliferation behavior.

Blood compatibility: To assess the blood compatibility and coagulation on the coatings, spider silk coated 316L stainless steel substrates were incubated with fluorescent fibrinogen enriched fresh human whole blood for 15, 30 and 60 minutes. Blood coagulation was analyzed by fluorescence readings, confocal and scanning electron microscopy.

Drug elution/enzymatic degradation: Drug elution profiles of sirolimus into phosphate buffered saline were recorded over 15-30 days, and degradation was assessed in a physiologically relevant mixture of enzymes.

RESULTS: All cell types exhibited poor adhesion to the unmodified recombinant spider silk. In contrast, all cell types illustrated robust attachment to the positive controls, spider silk functionalized with the RGD ligand (spider silk-RGD) and tissue culture plastic. Furthermore, human fibroblasts and smooth muscle cells showed minimal attachment to the spider silk-REDV material, while human endothelial cells adhesion was significantly elevated compared to the unmodified silk. Long term proliferation assays further illustrated the ability of the spider silk-REDV material to promote endothelialisation.

Whole blood coagulation assays showed significant blood coagulation on uncoated 316L stainless steel (a common stent material). However, all silk materials exhibited minimal blood coagulation, illustrating the haemocompatibility of these materials. SEM and confocal images further strengthened these findings. Enzymatic degradation and drug elution assays illustrate that the spider silks can be degraded by natural processes into natural amino acids and that clinically relevant drugs can be embedded in the materials to allow local drug delivery.

DISCUSSION & CONCLUSIONS: Spider silk is an interesting new coating material for cardiovascular stents, combining low attachment of cells and blood components with the selective adhesion of endothelial cells to form a monolayer of endothelium inside the stented blood vessel to protect the implant from the environment.

Keywords: Biomaterials, Cardiovascular

OP-081

Upscaling manufacturing of human induced pluripotent-derived mesenchymal stromal cells in a 1-l bioreactor system

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INTRODUCTION: Mesenchymal Stromal Cells (MSCs) are a population of heterogeneous cells that have emerged as primary candidates for cell therapy due to their immunomodulatory and reparative effects. Sourced from primary tissues, their inherent heterogeneity in addition to variables introduced during the manufacturing process can cause inconsistencies in MSC preparation, leading to unpredictable outcomes in clinical trials. The aim of this study was to establish the optimal conditions for the automated culture of human induced pluripotent stem cell derived MSCs (hiMSCs), with the goal of producing a more consistent MSC-based therapy that overcomes the heterogeneity of primary MSCs while also meeting the demand for large-scale cell manufacturing of clinical grade-MSCs.

METHODS: hiPSCs were differentiated to hiMSCs using STEMdiff™ mesoderm induction medium followed by expansion in a proprietary serum-free medium, Purstem2 (PS2). Generated hiMSCs were characterised using flowcytometry and tri-lineage differentiation to ensure compliance with ISCT standards. A stirred tank system was selected for expansion and several microcarriers were screened in static culture. Those that performed well were used for the culture of hiMSC in 100 mL spinner flasks, where cell growth and proliferation was measured daily. Several xeno-free dissociation reagents were also examined. Findings from the 100 mL cultures were translated to a 1L Bioflo 320 bioreactor in order to examine the large-scale expansion of the hiMSCs.

RESULTS: Generated hiMSC showed attachment to both plastic and coated microcarriers, however SoloHill® Plastic microcarriers were selected as the microcarrier of choice. hiMSC proliferation within 100 mL spinner flasks showed exponential growth of hiMSC cell lines, indicating that cells could proliferate well within a 3D environment under xeno-free conditions. The release of hiMSCs from the microcarriers was also examined resulting in the selection of TrypZean as the reagent most suitable for detachment as hiMSCs were removed from the microcarriers in approximately 10 minutes. Scale up to a 1L level was then investigated using a 1L Bioflo 320 bioreactor.

DISCUSSION & CONCLUSIONS: STEMdiff™ mesoderm differentiation followed by expansion in PS2 was successful in generating hiMSCs in compliance with ISCT standards. Expansion of these cells in 100 mL stirred flask cultures have shown that these hiMSCs could be cultured in a scalable 3D environment under xeno-free conditions. Translation to the 1L Bioflo 320 bioreactor had promising results suggesting large-scale manufacturing is possible, however further work is needed to separate the cell product from the microcarriers.

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Keywords: Scaling up & manufacturing, Multipotent (mesenchymal) stem cells

OP-082

Searching for donor-independent metabolic markers by NMR metabolomics

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INTRODUCTION: Due to their ability to differentiate into several cell lineages and secrete bioactive factors crucial for tissue repair, mesenchymal stem cells (MSCs) are promising tools in tissue engineering for biomedical applications [1,2]. However, inter-donor variability limits the clinical value of MSCs, triggering attempts to use data signatures as predictors of differentiation potential [3].

METHODS: Here, an untargeted nuclear magnetic resonance (NMR) metabolomics time course characterization of human adipose mesenchymal stem cells (hAMSCs) endometabolic profile was carried out for different donors with different osteogenic and proliferation capacities, to identify donor-dependent and donor-independent metabolic signatures of each process.

RESULTS: A set of metabolic adaptations were descriptive of proliferation, independently of the donor, including changes in alanine, aspartate, proline, tyrosine, ATP, ADP and plasmalogens. An osteogenic-specific donor-independent signature was also suggested, mostly involving alterations in the levels of taurine, glutathione, methylguanidine, adenosine, inosine, uridine, sphingomyelin, phosphatidylcholine, creatine/phosphocreatine, choline/phosphocholine and ethanolamine/phosphocholine.

DISCUSSION & CONCLUSIONS: Overall, the present work revealed the great potential of NMR metabolomics to monitor the dynamic metabolism of MSC osteodifferentiation, ultimately allowing the possible discovery of universal metabolic markers of proliferation extension and osteogenic efficacy, with potential translation to in vivo clinical practice.

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Keywords: Multipotent (mesenchymal) stem cells, Other

OP-083

When stochasticity meets precision: Using single cell genomics to refine cell therapies in bone and cartilage repair

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INTRODUCTION: Despite osteoarthritis (OA) representing a large burden for health and social care systems, there is no effective intervention capable of regenerating the damaged joint cartilage in OA and current clinical management for end-stage disease remains joint replacement surgery. Mesenchymal stromal cells (MSCs) are adult-derived, multipotent cells which are a candidate for musculoskeletal cell therapy. However, their precise mechanism of action remains poorly understood.

METHODS: The effects of an intra-articular injection of human bone marrow derived MSCs into a mouse knee osteochondral injury model were investigated in young C57Bl/6 mice, aged C57Bl/6 mice and GNL3 heterozygote mice. Tissue repair was assessed using histology and imaging, and cage activity monitoring was used to evaluate recovery. The cell therapy was retrieved from the mouse knee at different time points and single cell RNA sequencing was performed to elucidate the transcriptomic changes relevant to driving tissue repair, and changes in the mouse immune cell populations were characterised using mass cytometry.

RESULTS: Histological assessment reveals that MSC treatment is associated with improved tissue repair in young C57Bl/6 mice but is more limited in aged C57Bl/6 mice and GNL3 heterozygote mice. Cage activity monitoring suggests that MSC treatment is also associated with significantly improved recovery following injury. Single cell analysis of retrieved human MSCs showed spatial and temporal transcriptional heterogeneity between the repair tissue (in the epiphysis) and synovial tissue. A transcriptomic map has emerged of some of the distinct genes and pathways enriched in human MSCs isolated from both tissues following osteochondral injury. Several MSC subpopulations have been identified, including proliferative and reparative subpopulations at both 7 days and 28 days after injury. Supported by the mass cytometry results, the immunomodulatory role of MSCs was further emphasised, as MSC therapy was associated with the induction of increased numbers of regulatory T cells correlating with enhanced repair in the mouse knee.

DISCUSSION & CONCLUSIONS: The transcriptomes of a retrieved MSC therapy were studied for the first time and their heterogeneity described. An important barrier to the translation of MSC therapies is this lack of understanding of the heterogeneity that exists, and therefore the lack of precision in its use. MSC subpopulations with different functional roles may be implicated in the different phases of tissue repair and the data offers insights into the interaction between the cell therapy and the host tissue. The safety and efficacy following xenotransplantation of human MSCs further supports the development of allogeneic MSC treatments in humans.

Keywords: Multipotent (mesenchymal) stem cells, Cartilage / joint and arthritic conditions

OP-084

Optogenetic Control of BMP Signalling to drive Human Pluripotent Stem Cell Chondrogenesis

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INTRODUCTION: Visible light is increasingly being used to control cellular function and physiology through a technology called optogenetics. Optogenetic tools typically consist of a photoreceptor chimeraised to a signalling molecule of interest to render its activity light sensitive. We recently generated an optogenetic Bone Morphogenetic Protein (BMP) system (optoBMP), which can be used to induce signalling activity upon blue light illumination. BMPs are a family of signalling molecules crucial in chondrogenic development, particularly in the generation of cartilaginous matrix. Chondrogenic differentiation of human pluripotent stem cells (hPSCs) currently relies upon supplementation of recombinant growth factors to activate TGF β /BMP signals, but this may lead to poor differentiation reproducibility and quality because of batch-to-batch variation and growth factor degradation. Optogenetic intervention will enable direct upregulation of chondrogenic processes and refined control of differentiation.

METHODS: The OptoBMP system consisted of modified BMPR1B/ALK6 and BMPR2 receptors, anchored to the membrane with a myristoylation domain at the N-terminus and a light sensitive Light-Oxygen-Voltage (LOV) sensing domain chimeraised at the C-terminus. An optoBMP system transgene was inserted into the AAVS1 locus of hPSCs through CRISPR/Cas9 gene editing (optoBMP-hPSCs). Supplementation of BMP growth factors within an hPSC chondrogenic differentiation protocol was replaced with an optimised regime of blue light stimulation. The effect of optogenetic manipulation upon hPSC chondrogenic differentiation was characterised through protein and transcriptional analyses.

RESULTS: Modified optoBMP-hPSCs expressed optogenetic receptors and recapitulated activation of canonical BMP signalling mechanisms upon blue light illumination. RNAseq and qPCR analyses indicated that optoBMP activation could drive hPSC chondrogenic differentiation through a BMP signalling mechanism, resulting in significant upregulation of known BMP transcriptional targets (ID1-4), key chondrogenic genes (SOX5,6,9, COL2A1, ACAN) and enrichment of GO terms associated with developmental chondrogenesis (skeletal system morphogenesis, cartilage development). Additionally, optogenetic manipulation resulted in significantly higher expression of some chondrogenic genes when compared to BMP growth factor addition, including SOX5,6 and COL2A1. Furthermore, pre-chondrogenic cells generated through optoBMP stimulation produced a hyaline-like cartilaginous matrix when cultured in pellets, which were rich in glycosaminoglycans, type II collagen and aggrecan.

DISCUSSION & CONCLUSIONS: Our findings indicate that the optoBMP system can be utilised to drive chondrogenic differentiation of hPSCs in lieu of BMP growth factors. Furthermore, generation of a hyaline-like matrix indicates that optogenetic-mediated signal transduction through BMPR1B is a promising approach to tissue engineering of hPSC-cartilage constructs. We aim to combine this system with biofabrication technology and optogenetic control of other signalling pathways to generate layered and complex skeletal constructs.

Keywords: Biologics and growth factors, Embryonic stem cells

OP-085

Harnessing cell-material interactions to control stem cell secretion for osteoarthritis treatment

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INTRODUCTION: Owing to their capacity to secrete immunomodulatory factors in response to a pro-inflammatory environment, mesenchymal stem cells (MSCs) have been considered as therapeutic cells to treat osteoarthritis (OA). Unfortunately, intraarticular MSC therapy is limited by poor cell survival, potential cell leakage out of the joint, and a lack of control over cell fate. Combining biomaterials, especially hydrogels, with MSCs has emerged as a promising approach to improve MSC delivery and therapeutic efficacy; however, the influence of the composition and mechanical properties of hydrogels on the immunomodulatory ability of encapsulated MSCs is still largely unknown. In this context, we endeavored to better understand the relationship between cell-material interactions and MSC secretion in order to design a new material-assisted MSC therapy of OA.

METHODS: First, we developed a new injectable hydrogel platform allowing us to independently tune the composition (i.e., polymers and biomimetic peptides) and mechanical properties (i.e., stiffness, viscoelasticity) of hydrogels. Then, we used this platform to study the effects of the composition and mechanics of hydrogels on encapsulated MSC secretion. This allowed us to identify a formulation able to maximize the secretion of a variety of factors of interest by encapsulated MSCs. We evaluated the ability of MSCs encapsulated in the selected hydrogel formulation to trigger macrophage polarization toward an anti-inflammatory phenotype *in vitro*, and finally evaluated the therapeutic potential of the new hydrogel-assisted MSC therapy in rabbit model of OA.

RESULTS: We successfully developed both hyaluronic acid (HA)-based and alginate-based hydrogels that are stable long-term, either viscoelastic (relaxation time $\approx$ 1 second) or purely elastic (relaxation time $>$ 1000 seconds), and that can be modified with chondroitin sulfate or biomimetic peptides (i.e., RGD, HAVDI). Encapsulated MSCs showed excellent viability ($\geq 95\%$) after 7 days in all of the hydrogels, validating the use of the new hydrogel platform. Investigating the effects of cell-material interactions on MSC secretion, we showed that viscoelastic hydrogels composed of hyaluronic acid uniquely increase the MSC secretion of immunomodulatory factors (e.g., IDO, PGE2, IL-4, FGF). These hydrogels promoted the polarization of macrophages toward an anti-inflammatory (M2) phenotype, and showed a trend toward an improved OARSI score in a rabbit model of OA.

DISCUSSION & CONCLUSIONS: Overall, this study put into perspective the effects of hydrogel mechanical and biochemical properties on the MSC secretion in a pro-inflammatory environment. We showed that viscoelastic HA-based hydrogels are suitable candidates to increase MSC immunomodulatory properties, with the ability to enhance the MSC therapeutic potential.

Keywords: Hydrogels and injectable systems, Cartilage / joint and arthritic conditions

OP-086

Towards creating a holistic urinary tract for regenerative medicine

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INTRODUCTION: Generating a functional kidney and a lower urinary tract from human pluripotent stem cells (hPSCs) could revolutionise the treatment of kidney failure. This condition affects three million people worldwide. We showed the feasibility of driving hPSCs to differentiate into glomeruli and tubules that became vascularised after implantation into severe combined immunodeficiency (SCID) mice (1, 2). These tissues, however, lack a ureter, ruling out the possibility of a holistic urinary tract.

METHODS: We hypothesised that chimeric kidneys could be made by combining hPSC-derived nephrons with native embryonic ureters. We differentiated human embryonic SCs towards the nephron lineage in 2D culture and transferred these cells into 3D culture. In some cases, we inserted an embryonic mouse ureter into the cell mass. We then studied organoids in culture or after implantation into SCID rat thighs where they were placed inside a perforated chamber containing an arterio-venous loop (AVLs) fashioned as we described (3). Serial sections cut through harvested tissues were analysed with markers of nephrons, urothelia and smooth muscle.

RESULTS: As expected, cultured organoids derived from hPSCs alone contained glomeruli and tubules. In contrast, chimeric organoids contained nephrons around a central ureter tube that had epithelial and smooth muscle components. Quantification showed that nephrons in cultured organoids were more plentiful in chimeric tissues. In other experiments, implanted organoids were harvested after four weeks. AVLs contained an arterial arm and an arterialisised venous arm, with numerous small blood vessels sprouting from the latter structure. Implanted chimeric organoids contained a ureter tube from which branch-like structures emanated near hPSC-derived nephrons. Nephron tubules were found in both chimeric and non-chimeric organoids but they appeared larger in the former. Both latter observations suggest cross-talk between nephrons and the ureter tube.

DISCUSSION & CONCLUSIONS: Thus it is possible to create compound viable tissues containing hPSC-derived nephrons and native ureters. Moreover, we conclude that the presence of a ureter may stimulate the growth and differentiation of adjacent nephrons and vice versa. Further work is required to determine both whether the lumens of nephron tubules join with the ureter to form a potential functional unit, and which molecules are involved in paracrine signalling between these tissues within chimeric organoids.

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Keywords: Embryonic stem cells, In vivo and animal models

OP-087

Paracrine functionalities of hiMSCs cultured in clinical grade system: in vitro biological validation of hiMSC-derived EVs for the treatment of osteoarthritis

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INTRODUCTION: Mesenchymal stem cells (MSC)-based therapies have been identified as promising approach for treatment of osteoarthritis (OA). Research in the last decade strongly suggests that MSC-mediated therapeutic benefits are mainly due to their secretome, among the factors responsible for the cell paracrine effects, extracellular vesicles (EVs) have been recently described as new players in cell-to-cell communication. It is suggested that MSC from younger donor sources compete with adult MSC in their EV production capabilities. Therefore, there is a great interest to develop MSC generated from induced pluripotent mesenchymal stem cells (iMSC) to provide a promising cellular source for the manufacture of EV products. In this study, we isolated and tested the efficacy of EVs secreted by MSCs and by iMSC on the treatment of OA in vitro

METHODS: To obtain high-quality EVs, we optimized the culture conditions of MSCs and hiMSC, the supernatant collection time, and EVs extraction methods. MSCs and iMSCs were culture in vitro in serum-free clinical grade condition. The cells, during the long term-expansion were characterized for surface expression pattern, proliferation ability, senescence rate and differentiation capacity. The culture media were collected continuously during the cell expansion, and EVs were isolated using ultracentrifugation. Nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and western blots, and non-conventional flow cytometry were used to identify EVs. We evaluated the biological effects of MSC and iMSC-derived EVs on IL-1 α treated human chondrocytes, to mimic the OA environment.

RESULTS: To achieve our objectives and generate novel therapies for modulation of established OA, we compare the therapeutic effects of EVs released by BM-MSCs and hiMSC. Generated hiMSC can be expanded in culture over an extended period without losing their typical fibroblastic morphology and their capacity for osteogenic and chondrogenic differentiation. Moreover, hiMSC-EV samples show biological activity in suppressing secretion of pro-inflammatory IL-8, IL6 by chondrocytes, even if prepared from conditioned media of late passaged cells. While the MSC control, drastic decline the proliferation, differentiation, and function after 3 in vitro passages. Further analyses such as microRNA and immunomodulatory activity are underway to evaluate the therapeutic role of MSCs and hiMSCs-derived EV.

DISCUSSION & CONCLUSIONS: The focus of our study was to develop and characterize human iMSC to assure sustainable, reproducible and validated cell sources for the production hiMSC-EV off-the-shelf products as a future therapeutic agent for OA patient cohorts.

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Keywords: Extracellular vesicles, Cartilage / joint and arthritic conditions

OP-088

Generation of 3D models of vascularized tissue in a millifluidic optically-accessible bioreactor

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INTRODUCTION: Unraveling the physiological reactions induced by biomaterials implantation is an essential requirement before clinical translation. Integration of tridimensional cell culture systems and computational studies can help modelling the behavior of living tissues [1]. By combining computational fluid dynamics (CFD) analyses with the use of a millifluidic optically accessible bioreactor (MOAB), suitable for dynamic culturing of cells in a microscopic tridimensional (3D) environment, we produced a quick and reliable technique for modelling vascularized tissue regeneration "in vitro".

METHODS: We fabricated 3D micro scaffolds consisting of two rows of nine microgrids, each of 500x500x40 μm , by 2-photon polymerization of the SZ2080 biocompatible photosensitive resin [2]. The geometry is a 3D micro grid characterized by pores of 50x50x20 μm^3 . We compared cell proliferation with/without micro scaffolds, and we investigated the system in perfused conditions, by comparing the micro scaffold to a flat glass coverslip at the bottom of the MOAB chamber. CFD analyses allowed to set-up optimal parameters for dynamic cell culture and shear stress values acting on cells cultured in the micro scaffold. Endothelial cells and fibroblasts seeded on glass coverslips and microstructures were imaged by confocal laser scanning microscopy (CLSM) for evaluating their proliferation rates and spatial organization. Specific growth factors (VEGF and TGF- β 1) were administered for stimulating vessels formation.

RESULTS: CFD analyses were performed for determining the optimal flow rate (10 $\mu\text{l}/\text{min}$) for achieving a maximum shear stress value of 0.1 mPa inside the scaffold. CLSM imaging showed that microstructures do not affect cell viability. Cocultures proliferated both in static and dynamic conditions. Moreover, upon administration of growth factors, we observed the coculture reorganization starting from day 4.

DISCUSSION & CONCLUSIONS: CFD analyses allowed to evaluate optimal parameters for cell culture in perfused conditions. Experimental results show that the scaffold and bioreactor design supported endothelial cells and fibroblasts growth. Our culture system is a quick and reliable tool for modelling vascularized tissue regeneration "in vitro". Co-cultures are ongoing to quantify vessels and connective tissue formation. Administration of growth factors will be performed for understanding their cellular uptake/release kinetics.

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Keywords: Bioreactors,

OP-089

Investigation of the mechanobiological regulation of bone regeneration within scaffolds in large bone defects comorbid with Type 2 Diabetes

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INTRODUCTION: The treatment of large bone defects remains a clinical challenge which gets even more challenging when comorbid with Type 2 Diabetes (T2D). T2D is a metabolic disease characterized by elevated levels of glucose that is associated with reduced bone regeneration and increased risk of fracture. Scaffolds have a high potential in the treatment of large bone defects by guiding bone regeneration through biomechanical cues[1]; however, the mechanobiological mechanisms behind bone regeneration within scaffolds in T2D remains largely unknown. This study aims to investigate the mechanobiological regulation of bone regeneration within scaffolds in a large bone defect comorbid with T2D.

METHODS: An in silico approach that combined finite element (FE) analysis, to determine the mechanical environment, and agent-based models (ABM), describing the biological processes, was used to investigate the bone regeneration within the scaffold in healthy and T2D rats [2]. Gyroid scaffolds were inserted into a large bone defect in a rat femoral osteotomy model, replicating an experimental setup. Scaffold pores were initially filled with granulation tissue, while PCL material properties were assigned to the scaffold. In the FE models, the T2D was under higher external mechanical loading (healthy: 325 g body weight; T2D: 560 g of body weight). In the ABM, cellular activity in healthy was described using previously described models [3] while in T2D several cellular activities were altered according to findings reported in the existing literature.

RESULTS: Mechanical strains were considerably higher in the T2D model. The healing outcome was substantially different between the healthy and T2D. In the healthy case, the bone formed at the walls of the scaffold, similar to in vivo observations while the T2D model showed reduced bone formation comparing well with the in vivo data

DISCUSSION & CONCLUSIONS: Computer model prediction results could explain the reduced bone regeneration in T2D within scaffolds as observed in vivo. This is in accordance with experimental studies showing reduced healing outcomes in T2D. Future studies will focus on further validating the developed model and the optimization of the scaffold design.

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Keywords: Diabetic healing, Biomaterials

OP-090

A validation method of the FE model for the design of bone tissue engineering scaffolds

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INTRODUCTION: Computer modelling is applied to a wide range of engineering applications to predict the outcome of a physical system. In the field of bioengineering, additive manufacturing (AM) technologies and computer modelling can be coupled to fabricate tissue engineering (TE) scaffolds and to study their behaviour under given circumstances (e.g., the effect of pore size, their shape, and degree of porosity on the mechanical properties of a TE scaffold under given loads).

METHODS: This work aimed to prepare a numerical tool for designing bone TE scaffolds. The mechanical behaviour of scaffold structures was studied by varying the pore size and the degree of porosity. To find a reliable model, the results of finite element analysis (FEA) were validated against the experimental data. All the porous structures used for FEA validation were fabricated via stereolithography (SLA) using commercial photo-curable resin. This technique was chosen since it is able to produce prototypes with high resolutions and fine features. Fabricated specimens were subjected to morphometrical analysis using micro-CT, then mechanically tested.

RESULTS: First, it was possible to control the printability of the structures in terms of pore size and degree of porosity, understanding the printing limitations due to the mechanical properties of the material (i.e., viscosity and stiffness). Moreover, it was possible to obtain open-porous structures from the viewpoint of obtaining high cell proliferation in the scaffold.

Secondly, it was revealed that the FEA results showed good agreement with the experimental results, which is evidence for the successful validation of the developed model. Much effort was put toward studying functionally graded (FG) structures (i.e., structures showing gradients of porosity along at least one material direction) since they allow tuning the stiffness of the scaffold structure by varying position in space (e.g., mimicking the gradient change of elastic modulus in human bone).

DISCUSSION & CONCLUSIONS: From the design point of view, the developed FE model enables for correlating the internal architecture with the final mechanical properties of the FG structure, being able to predict the elastic region of the stress-strain curve.

The obtained data are a starting point for future works. The ultimate aim is to expand the developed FE model to consider non-linear phenomena related to specific material properties and large deformations.

Keywords: Additive manufacturing,

OP-091

A shareable and replicable design for a perfusion bioreactor: decisions on 3D printing supported by a numerical modelling framework

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INTRODUCTION: Many bioreactor designs, shapes and functions have been proposed in Tissue Engineering (TE) to better support the growth and proliferation of particular cell populations. Some of those designs aim to include a specific set of sensors or actuators to allow monitorization of the in vitro environmental conditions or add specific physical differentiation promoters (e.g. electric field or mechanical stimuli) for a particular cell line. Usually, bioreactor design developments are not shared open-source with proper fabrication and integration instructions, allowing easiness of reproducibility and usage by the TE research community. We aim to present a bioreactor design easily replicable and flexible enough to adapt to each particular need in the context of TE research.

METHODS: A multistep bottom-up approach to design a perfusion bioreactor was conducted with all design decisions supported by a finite element numerical model. The single-phase fluid laminar flow or electric currents physics interface were applied to guide the decision process using the results predicted by each model. Bioreactor components were conceived in a sequential and iterative process, alternating CAD design with finite element analysis. All bioreactor parts were designed to be manufactured in a commercial fuse filament fabrication 3D printer. Hardware and firmware were developed to control perfusion pumps and a Peltier plate heater based on Arduino™ microcontroller architecture and some standard power controller circuits. Bioreactor fabrication and the validation of the perfusion flow were also performed.

RESULTS: A complete perfusion bioreactor design was achieved with the capability to sustain 4 scaffolds per bioreactor and provide multiple solutions for mechanical or/and electric stimulation delivery. This bioreactor will be made available in an open-source platform with its precise fabrication instructions, material lists, source files, and developed firmware. The complete bioreactor numerical model, “digital-twin”, will also be made available on the same platform. The open-source design and model-fabrication framework provided here will empower researchers with larger flexibility to adapt the current design to new TE goals and integrate new development ideas while simplifying their approach through a shared open-source platform.

DISCUSSION & CONCLUSIONS: Despite multiple designs available in the literature, the need for a shared bioreactor platform with high customization potential, with methods easy and affordable to reproduce, remains challenging. The proposed bioreactor methodology of design, fabrication and open-source sharing aims to help and motivate TE researchers to further enhance bioreactor technologies' adoption, increasing its replicability and evolution. Also, it highlights the importance of having a digital model guiding the TE experimental work.

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Keywords: Additive manufacturing, In silico models

OP-092

Computer-aided scaffold design optimization towards enhanced bone regeneration

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INTRODUCTION: Large bone defects remain a clinical challenge, with a gold standard treatment – autologous bone graft – that presents many drawbacks. Design optimized scaffolds appear as a promising alternative [1]; however, bone scaffold design remains a trial and error approach where some specific properties (porosity, mechanical properties, etc) are individually optimized. Thus, the aim of this study was to develop a computer-aided scaffold design optimisation framework towards enhanced bone regeneration, taking into account the dynamics of the bone regeneration process.

METHODS: A computer model of scaffold-guided bone regeneration was developed and tested against different experimental setups that have used different scaffold designs for large bone defect healing in large animal models [2,3]. The model takes into account the scaffold design (architecture and material properties) as well as its interaction with different cellular processes (e.g. migration). Computer model predictions of bone tissue formation within the scaffold pores were compared against in vivo experimental data. The validated model was then used to develop a computer framework that allow us to optimize the scaffold design with the objective of achieving maximum bone regeneration.

RESULTS: The computer model of scaffold-supported bone regeneration is able to explain experimental observations of bone tissue formation within a honeycomb titanium scaffold and a strut-based PCL- β TCP scaffold. In both experimental settings, scaffold surface guidance was predicted to play a key role on the regulation of cellular activity. Computer-aided optimization resulted in a scaffold design which was predicted to achieve almost complete bone regeneration within a large bone defect.

DISCUSSION & CONCLUSIONS: We have developed a framework that allows 1) to investigate the mechanisms behind scaffold-supported bone regeneration and 2) to optimize the scaffold design to achieve maximum bone regeneration. Although, the computer model of bone regeneration has shown promising results in different experimental settings, further testing of the model and validation against experimental data is needed to ensure model robustness. The optimization framework allows shape optimization of specific scaffold designs. Future studies will aim to include computer tools for topological scaffold design optimization. Moreover, experimental validation of the optimization framework will be performed.

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Keywords: Bone and bone disorders (osteoporosis etc), Biomechanics / biophysical stimuli and mechanotransduction

OP-093

High-Resolution Gyroid Bone Scaffold Manufactured via Digital Light Processing 3D Printing

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INTRODUCTION: Digital light processing (DLP) is a 3D printing method that can create a complex structure with fine details that typical extrusion-based 3D printing cannot provide. This allows the fabrication of biomimetic architecture, such as the gyroid, that mimics the cancellous bone structure and has a continuous surface suitable for cell attachment. However, cytocompatible resins for DLP 3D printing are still limited. Here we report the fabrication of a gyroid scaffold by DLP 3D printing of an isosorbide-based photopolymer.

METHODS: Isosorbide-based photopolymer, CSMA-2, was synthesised via 2-step reactions. CSMA-2 rheological properties and printability were then evaluated. Gyroid scaffolds were 3D printed by the DLP method, followed by a post-curing process. The 3D printing resolution, surface morphology, water contact angle, and mechanical properties were analysed. In vitro 3D cell culture was performed to observe the cytocompatibility of the 3D-printed gyroid scaffold.

RESULTS: CSMA-2 showed Newtonian behaviour with a viscosity value of 0.3 – 0.5 Pa.s, which is suitable for DLP 3D printing. Biomimetic gyroid scaffolds were successfully fabricated by 3D printing CSMA-2 via DLP. The 3D printed layer thickness corresponded to the 3D printing resolution set by the 3D printer. Surface morphology and water contact angle (<80°) were favourable for cell attachment. The compressive modulus of the 3D-printed gyroid scaffold (0.43 – 0.54 N/mm²) was similar to the human cancellous bone compressive modulus. Cells were found attaching, proliferating, and differentiating on the gyroid scaffolds during incubation.

DISCUSSION & CONCLUSIONS: Isosorbide has been reported to have optical properties suitable for light-based 3D printing and can improve the mechanical properties of polymers due to their bicyclic structure. CSMA-2 also demonstrated these advantages. It allowed 3D printing of intricate gyroid architecture via DLP with a resolution of up to 25 micrometres and can print faster than other light-based 3D printing methods. The 3D-printed gyroid scaffold showed favourable mechanical and

biological properties for bone tissue engineering and patient-specific implant.

Keywords: Biomaterials, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-094

Microfluidic 3D biofabrication for the patterning of hierarchical gradient constructs

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INTRODUCTION: Functionally graded materials (FGMs) are ubiquitous in human tissues.

Nevertheless, there has been limited attempt in replicating FGMs in 3D to generate constructs for tissue engineering and regenerative medicine purposes [1,2]. Here, we describe the fabrication of FGMs harnessing oil-in-water (o-w) and water-in-water (w-w) emulsions using a microfluidic flow-focusing printhead and a fluid-gel support bath, to engineer hierarchically functional implants.

METHODS: An organic (cyclohexane) or biocompatible dispersed phase was fractioned in a continuous (Dextran, gelatin, hyaluronic acid methacryloyl (DexMA, GelMA, HAMA)) phase in a flow-focusing microfluidic printhead. Physico-chemical analysis was carried out using scanning electron microscopy (SEM), optical coherence tomography (OCT) and dynamic mechanical analysis (DMA). 3D deposition of FGMs was carried out in an agarose fluid-gel previously described [3]. Human bone marrow stromal (HBMSCs), vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2) were encapsulated either in the dispersed or the continuous phase of the w-w emulsion, printed and implanted in a chick chorioallantoic membrane (CAM) model.

RESULTS: Deposited o-w and w-w fibres were tailored in porous content depending on the ratio of dispersed and continuous phase flows. On-chip characterisation revealed a significant proportionality ($p < 0.0001$) between the diameter of the droplets and the volume fraction of the dispersed phase. Physico-chemical characterisation (SEM, OCT, DMA) of the printed scaffolds revealed hierarchically distributed porosity and preserved architecture following 3D deposition. DexMA, GelMA, HAMA were independently 3D printed comprising a variable set of porous density gradient. HBMSCs were printed when included either in the dispersed or the continuous phase, revealing no significant difference in viability post-printing. VEGF and BMP-2 included in the dispersed phase and patterned in 3D, elicited a tuneable release according to the size and density of the encapsulating droplets. HBMSCs were found functional upon stimulation with VEGF or BMP-2 following implantation in CAM, demonstrating the ability to produce readily implantable skeletal constructs.

DISCUSSION & CONCLUSIONS: FGM precursors (o-w, w-w) did not influenced the dispersion formation characteristics and reproducibility, offering a rapid and functional platform for the fabrication of hierarchical constructs. Altogether, we highlighted the synergistic combination of microfluidic technology with a bioprinting platform for the patterning of FGMs, demonstrating the engineering of a biphasic system particularly relevant for the control of densities during the biofabrication of tissue substitutes and implants.

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Keywords: 3D printing and bioprinting, Biofabrication

OP-095

Optic Nerve Neuroprotection and Axon Regeneration using Matrix-Bound Nanovesicles and Fluvastatin in Glaucoma and Optic Nerve Trauma Models

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INTRODUCTION: Optic nerve injuries arising from glaucoma or traumatic injury result in irreversible loss of vision. The optic nerve (ON) is an extension of retinal ganglion cell (RGC) axons, and damage to the ON results in RGC death, axon and vision loss. There are currently no effective treatments to repair axon damage. We investigate the combination of two therapies for glaucoma and ON trauma: Matrix-bound nanovesicles (MBV) that represent extracellular vesicles embedded in the extracellular matrix, and Fluvastatin, an FDA-approved cholesterol-lowering drug.

METHODS: We examine the ability of local intravitreal treatment with MBV and Fluvastatin to prevent RGC death and axon degeneration in three models of ON damage: (1) glaucoma model- elevated intraocular pressure (rat), (2) trauma model- ON crush (mouse), (3) a large animal, clinically-relevant trauma model- standard and enhanced ON crush (pigs). In all models we examine RGC number and length of axons with a combination therapy of MBV and Fluvastatin injected intraocularly immediately and two and/or three times after injury.

RESULTS: In the glaucoma model, MBV treatment prevented loss of RGCs, and increased axon length compared to PBS controls. In the mouse model of ON trauma, MBV and Fluvastatin treatments increased RGC survival and number, and length of axons, more so than either MBV or Fluvastatin alone. Preliminary findings from the porcine model of ON trauma confirmed that ON crush injury can be induced reliably and reproducibly with negligible complications. We also established a novel technique of monitoring visual evoke potentials and implemented an FDA approved device for sequential ERG monitoring to assess functional visual outcomes following neurotherapeutic interventions in ON crush injury. Results of RGC numbers and axon lengths in porcine models of ON trauma are currently being evaluated and will be presented.

DISCUSSION & CONCLUSIONS: MBV and Fluvastatin represent a novel therapy for neuroprotection and axon regeneration in glaucoma and traumatic ON injury models.

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Keywords: Extracellular vesicles, Drug delivery

OP-096

On-chip platform for derivation of ocular cells from hiPSCs

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INTRODUCTION: Vision loss due to ophthalmic diseases affects a large portion of the human population, decreasing quality of life. Current regenerative medicine approaches in ophthalmology have been significantly influenced by iPSCs and their potential in ocular disease modeling and treatment of ocular diseases. Dynamic culture conditions have been established that allow cell differentiation and tissue growth rather than using animal models to improve the in vitro models. This study aims to generate a microfluidic platform to understand microphysiological features of hiPSC-derived anterior part of the eye, such as mechanotransduction.

METHODS: In our previous study, hiPSCs were differentiated to multi zonal ocular cells (MZOCs) to recapitulate human ocular structures adapting the protocol developed by Li and colleagues.^{1,2} We performed the bulk RNA sequencing to understand the transcriptional profile of hiPSC-derived ocular cells and found the mechanobiology related genes. Differentiation and growth of the hiPSCs were performed in engineered microfluidic platforms to further understand the mechanotransduction. Both ocular cell differentiation and mechanotransduction were characterized at mRNA and protein levels. Also, in order to analyse the metabolomic phenotype of samples cultured under the dynamic conditions were collected.

RESULTS: We observed that multi-zonal cells display ocular expression patterns, suggesting self-formed multi-zonal cells show capacity to form ocular organoids. The transcriptomic profiles showed that 12 and 15 genes related to eye development and mechanotransduction, respectively. Differentiated ocular structures display limbal epithelial stem cells, periocular mesenchymal cells, lacrimal gland myoepithelial cells and cornea epithelial cells. The key markers of mechanotransduction YAP/TAZ and Piezo1 were found to expressed in the samples from direct method and gradually decreased in transferred to chip method according to size effect. Metabolomic analysis showed that 247 of all 1778 metabolites them were identified by using databases. Also, metabolites from the both 96- and 48-well plate-size chips showed distinct grouping at day 7.

DISCUSSION & CONCLUSIONS: With the main focus on recapitulating the cell architecture by combining cell culture techniques with microfluidics have great potential to understand of

mechanotransduction effects of ocular structures. The findings will enable the development of different perspectives on new approaches to be used in treatment of ocular diseases. However, OMICS can provide the information for ocular differentiation and disease models.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Induced pluripotent stem cells

OP-097

High-throughput repurposing of discarded abattoir waste: simultaneous opportunities for tissue engineering, regenerative medicine, and circular economic sustainability

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INTRODUCTION: Corneal disease is a leading cause of blindness globally that stems from various etiologies. High-throughput platforms that can generate substantial quantities of corneal grafts will be invaluable in addressing the existing global demand for keratoplasty. Slaughterhouses generate substantial quantities of underutilized biological waste that can be repurposed to reduce current environmentally unfriendly practices. Such efforts to support sustainability can simultaneously drive the development of transplantable ovine corneas and patient-specific bioartificial keratoprotheses. **METHODS:** Over 100 discarded eyes from the prominent Arabian sheep breeds in our surrounding region of the United Arab Emirates (UAE) were repurposed to generate native and acellular corneal keratoprotheses. Acellular corneal scaffolds were created using whole-eye immersion/agitation-based decellularization with a widely available, eco-friendly, and inexpensive 4% zwitterionic biosurfactant solution (Ecover, Malle, Belgium). Conventional approaches like DNA quantification, ECM fibril organization, ocular transparency and transmittance, surface tension measurements, and Fourier-transform infrared (FTIR) spectroscopy were used to examine corneal scaffold composition and integrity.

RESULTS: Using this high-throughput system, we effectively removed over 95% of the native DNA from native corneas while retaining the innate microarchitecture that supported substantial light transmission (over 70%) after reversing opacity, a well-established hallmark of decellularization and long-term native corneal storage, with glycerol. FTIR data revealed the absence of spectral peaks in the frequency range 2849 cm⁻¹ to 3075 cm⁻¹, indicating the effective removal of the residual biosurfactant and cellular/tissue debris from the elute collected on the 2nd and 3rd day during the detergent removal process. Surface tension studies confirmed the FTIR data by capturing the progressive and effectual removal of the surfactant through tension measurements that ranged from approximately 35 mN/m for the 4% biosurfactant decellularizing solution, and approximately 50 mN/m, 60 mN/m, and 70 mN/m (comparable to pure water) in the elutes obtained from the 1st, 2nd and 3rd days after rinsing the scaffolds with saline, respectively.

DISCUSSION & CONCLUSIONS: To our knowledge, this is the first dataset to be generated outlining a platform that can produce dozens of native ovine xenografts and acellular corneal scaffolds that effectively preserve ocular transparency, transmittance, and ECM components using an eco-friendly

surfactant. Such native grafts may then be genetically modified to reduce rejection and retrovirus transmission in recipients. Analogously, decellularization technologies can support corneal regeneration with attributes comparable to native xenografts. Overall, this study presents a simplified, inexpensive, and scalable high-throughput corneal xenograft platform to support tissue engineering, regenerative medicine, and circular economic sustainability.

Keywords: Eye, Decellularised matrices

OP-098

Automated bio-fabrication of in vitro models of Glaucoma for high throughput testing

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INTRODUCTION: Glaucoma is currently the leading cause of irreversible blindness, the prevalence of which is set to increase by 74% in 2040. Current gold standard in vitro/in vivo models cannot fully replicate the complexity of glaucoma pathogenesis. Consequentially, current treatments into resolving the fundamental issues associated with glaucoma pathology (trabecular meshwork (TM) fibrosis) are limited and there has been little success in clinical trials of new treatments. Thus, there is a clear need for more high-throughput, reproducible, TM in vitro models to help overcome these issues. This study aims to create the first biomimetic TM in vitro model with tunable collagen fibre properties, aimed towards high throughput testing.

METHODS: Initially, type I collagenous constructs were formed through an automated gel-aspiration-ejection (GAE) method, with collagen fibre parameters and mechanics characterised. Thereafter, human trabecular meshwork cells (hTMC) patient samples were embedded in collagen hydrogels for further GAE processing. Metabolic activity was assessed, expression of TM protein markers (α - β -crystallin, elastin, α -smooth muscle actin, fibronectin and myocillin), and modulation of genotype (SNAIL1, TWIST1). Once characterised, 5ng/mL of transforming growth factor-beta2 (TGF- β 2) was introduced utilising the PODS[®] technology, a fibrotic response was estimated by quantifying pro-fibrotic protein expression (α -SMA, fibronectin and TGF β -2), elastic modulus and cytoskeletal markers.

RESULTS: The GAE method was observed to modulate collagen fibre parameters (increased collagen fibre density, anisotropy, decreased porosity), and elastic moduli in comparison to unmodified collagen hydrogel. Patient derived hTMC (n=3) cell density was standardised (100,000-200,000 cells/mL), prior to applying the GAE method. Enhancement of metabolic activity was noted within GAE constructs over a 5-day period, positive expression of all protein markers associated with the in vivo TM and upregulation of SNAIL1 and TWIST1 was observed. In addition, a fibrotic in vitro model was characterised with enhanced expression of α -SMA, fibronectin and intracellular TGF β -2, myofibroblast hTMC observed, and heightened stiffness.

DISCUSSION & CONCLUSIONS: Overall, modified collagen fibre parameters regulated the hTMC to express phenotypic and genotypic characteristics associated with the TM site of disease pathology in vivo. Moreover, a fibrotic in vitro model was induced by introducing exogenous TGF- β 2, creating a biological response similar to that found in glaucoma patients. To conclude, this study presents a first-of-its-kind in vitro TM model that can emulate in vivo functionality, being produced in an automated manner that has the potential for high throughput drug screening.

Keywords: Biofabrication, Eye

OP-099

Moving bio-fabrication towards the clinic – Precise Bio's Corneal Implant

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Belmaker
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INTRODUCTION: The field of Bioengineering made impressive strides in recent years, yet the commercialization of bio-fabricated tissues and organs is still a great challenge. Precise Bio has taken up the challenge and established an industry-oriented workflow for taking products into the market. The company is comprised of experienced biology, chemistry, physics, software, and engineering teams working together on advanced multidisciplinary projects.

METHODS: To achieve commercial biofabrication and the production of a clinically compliant product, a workflow integrating between chemical engineered robust scaffolds supporting bio-printing of biologically active tissues was established. The bio-fabrication automated platform is comprised of a combinational platform utilizing nano to pico-liter printing tightly controlled techniques, which allow precise deposition of cells within viscous bioinks, achieving high cell printing densities with over 95% cells viability. The bio-fabrication platform can be readily adapted to diverse tissues and organs. We present here one of Precise Bio's bio-fabrication technologies of a corneal graft.

RESULTS: Corneal endothelial cells disfunction lead to swelling of the collagen fibrils in the corneal stroma, leading to edema and blindness. This can be reversed using corneal transplantation. However, there is a worldwide shortage of high-quality corneal tissue, currently obtained exclusively from organ donors. To meet this shortage, Precise-Bio INC. developed an engineered corneal tissue substitute to replace damaged endothelial layers, called Precise Bio Endothelial Keratoplasty (PVEK). Human donor corneal endothelial cells are expanded under GLP conditions and later processed onto a bio-fabricated ultra-thin collagen scaffold, which has similar mechanical and chemical properties as the natural Descemet's membrane. These corneal implants contain high cell density >2500 cells/mm² and have shown preclinical robust efficacy in NZ rabbits', achieving baseline cornea thickness within 2-4 weeks post implantation, with high endothelial cell density. Importantly, the physicians describe the PVEK implant as an easy and friendly manipulable implant relative to the human cadaver donor tissues. Importantly, one donor may produce hundreds of PVEK corneal implants for keratoplasty transplantation, thus potentially alleviating the corneal shortage. Not only will the PVEK implant answer the shortage it will also provide well controlled (for cell density, identity, purity, potency) and safe (sterile) corneal implantable tissue.

DISCUSSION & CONCLUSIONS: Precise-Bio demonstrates here the ability to produce functional corneal endothelial implants for the clinical procedure of human keratoplasty under a strict quality-controlled process. Our platform can be readily extended to bio-fabricate other potential tissues and organs with the potential for upscaling towards industry-oriented commercialization.

Keywords: 3D printing and bioprinting, Biomaterials

OP-100

3D tissue-engineered in vitro models for the investigation of breast cancer progression

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INTRODUCTION: Current 2D breast cancer (BC) models fail to accurately model changes that take place within the extracellular matrix (ECM) as BC progresses, such as an increase in glycosaminoglycan (GAG) deposition, stiffness increase and metastasis via epithelial-mesenchymal transition (EMT). This highlights the need for the development of representative in vitro 3D models. Additionally, gene therapy such as microRNA delivery is an emerging area in BC research showing great promise as a potential treatment. This project aims to develop a scaffold model composed of matrix components of breast tissue, including collagen and GAGs; hyaluronic acid (HyA) and chondroitin sulphate (CS), known to be elevated in tumours (1), with stiffness comparable to cancerous breast tissue. These scaffolds will enable investigation of the role of ECM changes in BC progression and their potential use as a platform for gene therapy for manipulation of a specific microRNA of interest known to be overexpressed in patients with BC and associated with poor patient prognosis, and found to play a role in regulating EMT associated genes such as E-cadherin and vimentin in other cancer types.

METHODS: Collagen-based scaffolds comprised of varying concentrations of HyA or CS with stiffness comparable to cancerous and healthy breast tissue were fabricated using previously optimised protocols (2). Scaffold characterisation, biocompatibility and 3D cell migration were performed using SEM, porosity, mechanical testing, metabolic activity, DNA, cytotoxicity assays, qPCR, cytokine array and H&E staining, using triple-negative breast cancer (TNBC) cell line, MDA-MB-231. Nanoparticle optimisation was carried out to deliver a miRNA inhibitor to TNBC cells and determine the cellular effects.

RESULTS: All scaffolds were highly porous and biocompatible. Change in GAG concentration did not affect cell metabolic activity/proliferation or migration but altered the expression of our miRNA of interest and the expression of pro-inflammatory cytokines; an increase in HyA increased the cytokines while an increase in CS decreased them. Changes in ECM stiffness did not affect metabolic activity/proliferation but increased cell migration and gene expression of EMT genes, E-cadherin and vimentin. Nanoparticles also successfully delivered the miRNA inhibitor to TNBC cells and are under further investigation.

DISCUSSION & CONCLUSIONS: In conclusion, this study demonstrates the ability of collagen-based scaffolds to act as efficient platforms to investigate the role of changes in the ECM on breast cancer progression and as highly suitable models for gene therapy.

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Keywords: Cancer, Biomaterials

OP-101

The Nichoid Micro Scaffold as a Tool for Repurposing “Migrastatic” Drugs Exploiting Synthetic Lethality

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INTRODUCTION: Since the normal process of developing new drugs is long and expensive, in recent years the use of biological and medical big data for computer-based approaches has gained increasing attention to investigate new therapeutic possibilities for already approved drugs [1]. Of great interest is the exploitation of this approach in the field of therapeutic cancer research, and particularly the investigation of “migrastatic” agents able to contrast cell invasion and migration to prevent metastases formation [2]. Moreover, the use of synthetic lethality (SL) for developing drug targets could help to overcome resistance and side effects, leading to death only malignant cells carrying a specific mutation [3]. Here, we combined the computational integration of these three concepts with experimental drug testing on an innovative bioengineered micro-scaffold named Nichoid [4], able to mimic the structural niche of cancer cells adhering in 3D culture.

METHODS: We retrieved and integrated different data from several databases: DisGeNET for gene-disease association, SynLethDB for SL couples and DrugBank for drugs. We then selected two PARP-inhibitors and two statins to test on BRCA1-mutated ovarian and breast cancer cell lines. We expanded the cells on the Nichoid, a micro-structured substrate fabricated by two-photon laser polymerization in a biocompatible photoresin, and we compared the response of cells expanded on the Nichoid to cells cultured on conventional flat substrates.

RESULTS: Through a specific filtering scoring schema, we extrapolated a set of genes associated with metastases, the SL couples in which the sets are contained and we identified the drugs targeting SL partner genes. Among the repurposed drugs, we addressed statins, normally administered to lower lipid levels but with an ability to improve the response to anticancer therapies, according to retrospective studies.

Moreover, preliminary experimental results showed a marked difference in the effect of the PARP-inhibitor Olaparib on cells cultured on the Nichoid, compared to cells cultured on flat.

DISCUSSION & CONCLUSIONS: SL is an important frontier in cancer therapy of chemotherapy-resistant cancers. Computer-based approaches can help in the identification of potential new therapeutic couples to be tested in the context of SL strategies. Here, we show how crucial is to screen the new candidate drugs in realistic culture models, in which cells can respond to the drug as similarly as possible as in vivo.

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Keywords: Cancer,

OP-102

Biofabrication of a 3D-Malignant Melanoma *in vitro* model: One step closer to personalized cancer treatments

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INTRODUCTION: Conventional *in vitro* cancer models present considerable limitations such as the lack of a tumor microenvironment (TME), cell-cell and cell-extracellular matrix (ECM) interactions, and vascularization, which are crucial to recreate the tumor internal biology and to study the efficacy of treatments. 3D bioprinting technology is an excellent tool to overcome these limitations including the ability to recapitulate *in vitro* the patient's pharmacological response.

METHODS: Two three-layer multicellular models of malignant melanoma (MM) were bioprinted, composed of cancer stem cells (CSCs) isolated from an established MM cell line or a primary patient-derived cell line, fibroblasts, mesenchymal stem cells, and endothelial cells, embedded within an agarose-collagen type I hydrogel, recreating both TME composition and native melanoma tissue architecture. MM constructs features and functionality were characterized by different strategies, testing mechanical behavior, hydrogel structure, cell viability and metabolic activity, communication between cells, antitumor drug response, and *in vivo* tumorigenic potential of the hydrogels.

RESULTS: The embedded cells showed high proliferation, and metabolic activity and actively remodeled their TME. In addition, an inter-cell communication was established leading to a phenotypic switch and activation of the TME. The MM hydrogels showed skin-like rheological properties and were able to support an early onset of vascularization. Furthermore, MM hydrogels showed a different response to vemurafenib compared to cell cultures, and supported tumorigenesis in murine xenotransplants achieving more mimetic *in vivo* models, both in structure and composition, resulting in the formation of melanocyte nest-like structures in the apical layer of the construct.

DISCUSSION & CONCLUSIONS: A 3D bioprinted CSC-based human MM model has been developed that recreates both *in vitro* and *in vivo* TME and response to treatment. This MM construct constitutes a promising approach to better study *ex vivo* the complexity of dynamic interactions between tumor cells and surrounding stromal cells with ECM, and for the establishment of precision treatment regimens against MM.

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Keywords: 3D printing and bioprinting, Personalised medicine

OP-103

Decellularized patient-derived pancreatic ductal adenocarcinoma xenografts for tumour physico-chemical characterization

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INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive lethal malignancy and the third leading cause of cancer-related mortality in the USA. Recently, solid tumours like PDAC are being regarded as organ-like structures with a tumour microenvironment (TME) comprised of cancerous and non-cancerous cells, growth factors, and a tumoural extracellular matrix (tECM) (1). More research should focus on characterizing the tumour physico-chemical properties and its tECM as they may have an important impact on the success of cancer therapies. PDAC xenografts and their decellularized tECM offer a great source of material in terms of biomimicry with the original human tumour (1). Our first aim was to find a rapid and cost-effective ultrasounds-based method for routine decellularization of patient-derived PDAC xenografts. A second aim was to characterize the physico-chemical properties of the PDAC tumours and the tECM, which to the best of our knowledge has never been done before, in order to generate valuable information about the TME.

METHODS: To find the method for decellularization, we applied a factorial design of experiments (DOE). Assessment of decellularization quantified remaining cells using H&E-stained images and an algorithm. Next, we characterized the physico-chemical properties of the xenografts and tECM: architecture by histological stainings, scanning electron microscopy (SEM) and mercury intrusion porosimetry (MIP), permeability by MIP, elemental and molecular groups/chemical bonds footprints by energy dispersive X ray analysis (EDX) and Fourier transform infrared spectroscopy (FTIR).

RESULTS: Our DOE approach identified an optimal combination of factors for effective decellularization of PDAC xenografts. Eliminating cells opened the porous tECM network, which increased the permeability. The peaks/shoulders observed in the FTIR spectra corresponded to organic molecular groups bonds present in proteins, lipids and carbohydrates. As the cells were eliminated, new peaks and shoulders appeared.

DISCUSSION & CONCLUSIONS: Gradual cell elimination would make the tumour more permeable to infused drugs, large molecules, macromolecules, and therapeutic cells, which suggests a potential therapeutic strategy based on the therapeutic agent size. As cells are eliminated some molecular groups in the tECM become more available, which could be targeted in later stages of treatment for

complete removal of the tECM component of the tumour.

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Keywords: Cancer, Decellularised matrices

OP-104

Development of *vitro* models to assess glioblastoma multiforme migration using 3D bioprinting technology

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INTRODUCTION: Glioblastoma multiforme (GBM) remains the most lethal yet most frequent primary brain tumour, partially due to the highly aggressive migratory behaviour of GBM cells into inaccessible areas of the brain. Improved *in vitro* models are required to deepen understanding of migratory pathways for identification of drug targets, as well as better predicting drug outcomes *in vivo* by using innovative design to mimic native GBM microenvironments. Here we describe using multi-material 3D bioprinting technology for improved assessments of GBM migration.

METHODS: Cell lines – Human-derived glioma cell lines U87 and U251. Knockdowns of pro- and anti-migratory genes (ARHGAP29 and ARHGAP12, respectively) were also used to investigate the role of Rho GTPase activating proteins in glioblastoma migration. Spheroids were formed using low-attachment round-bottomed 96-well plates.

Migratory Assays – 2D migration assays were used to assess the effect of anti-migratory drugs Rhosin Hydrochloride and CCG-1423 on GBM cells in 2D. To assess GBM migration in 3D, a combination of cell-adhesive and non-cell adhesive biopolymers were patterned to guide GBM migration using an INKREDIBLE 3D bioprinter (CELLINK).

Confocal Microscopy - Green fluorescent protein (GFP)-tagged cell lines were used for improved visualisation of cells. F-actin (phalloidin-TRITC) staining was conducted after fixing spheroids with 4% PFA overnight, and visualising using confocal microscopy and Z-stacking techniques.

RESULTS: Substantial reductions in GBM migration were observed using a combination of anti-migratory drugs across migration assays. In these cells, a reduction of F-actin expression is observed using immunofluorescence staining. On the other hand, cells which are modified to promote migration (knockdown of ARHGAP12) showed an increase in actin expression.

DISCUSSION & CONCLUSIONS: The development of 3D models, particularly using advanced such as 3D bioprinting, is crucial to deepen understanding of migratory pathways in cancer - in this case understanding the role of Rho GTPases in GBM migration, specifically regarding actin expression and changes to cytoskeletal shape. These models allow the identification of drug targets (using knockdowns), as well as confirming drug action in 3D, more closely resembling GBM *in vivo*. These models, combined with advanced imaging techniques, may also be used for migratory assessments in other cancers to better predict patient outcomes and enhance the development of personalised medicine.

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Keywords: Biomaterials, Cancer

OP-105

A 3D tissue-engineered osteosarcoma model based on macroporous composite alginate scaffolds and perfusion

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INTRODUCTION: Tumor engineering utilizing certain strategies of tissue engineering has been recognized as a promising approach to creating reliable, long-term in vitro three dimensional (3D) tumor models that will capture key features of the in vivo tumor cell microenvironment attractive for cancer research, anti-cancer drug discovery and development of personalized medical treatments. The aim of the present study was to develop a 3D osteosarcoma model based on the use of macroporous alginate scaffolds with incorporated hydroxyapatite (HAP) particles imitating bone tissue extracellular matrix (ECM) and perfusion bioreactor providing enhanced mass transport and adequate shear stresses.

METHODS: Macroporous composites based on Ca-alginate hydrogel and commercially available HAP powder (2 wt.% alginate and 2 wt% powder) were produced by controlled alginate gelation followed by freeze-drying and partial rehydration in the culture medium. The scaffolds (~9.5 mm in diameter, ~4.5 mm thick discs) were then seeded manually with a suspension of murine K7M2-wt osteosarcoma cells (ATCC® CRL-2836™) in the culture medium (15x10⁶ cells cm⁻³ scaffold volume) and left for 24 h in the culture medium under static conditions. The scaffolds were further cultivated in a perfusion bioreactor ("3D Perfuse", Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia) at the continuous medium flowrate of 0.27 cm³ min⁻¹ (40 μm s⁻¹ superficial velocity) up to 7 days, while static cultures served as a control. The initial and cultivated scaffolds were examined regarding the cell metabolic activity (MTT assay), viability, morphology and distribution within the scaffolds by histological and scanning electron microscopy analyses.

RESULTS: The scaffolds were successfully seeded with the cells (efficiency >80%), which adhered to the pore surfaces and formed cell aggregates. After 7 days of cultivation, the cells in both static and perfusion cultures remained viable and metabolically active forming more compact cell spheroids. Still, the spheroids in the latter case contained higher amounts of ECM, especially reticular fibers, as revealed by the histological analysis. Also, immunocytochemical analysis has shown better oriented α-tubulin in perfusion cultures indicating stronger cell-cell interactions as compared to static cultures.

DISCUSSION & CONCLUSIONS: In vitro culture conditions based on biomimetic porous scaffolds and medium perfusion enhanced osteosarcoma cellular self-assembly into compact spheroids, thus showing potentials for utilization in studies of tumor development.

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Keywords: In vitro microenvironments, Bioreactors

OP-106

In vitro 3D osteosarcoma models of CSC-niche: optimization of serial sarcospheres and scaffold-based seeding as cancer-specific instructive microenvironment

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INTRODUCTION: Osteosarcoma (OS) constituted the main entity among primary bone sarcomas. The failure of conventional treatments is due to the presence of Cancer Stem Cells (CSCs). The CSCs are educated by the tumor ecosystem, which is composed by different cell types, an osteoid matrix and various environmental conditions. The microenvironment provides supportive cues to the CSCs which in turn modulate it to survive. Conventional in vitro 2D cell culture systems must be overcome to make room for advanced three-dimensional (3D) models as in vitro more predictive platforms. Promising results were obtained by our group in 2020, confirming the higher predictivity of in vitro 3D bone-like scaffold-based models of enriched-CSCs as sarcospheres¹ compared to scaffold-free conditions. However, the variability of serial sarcospheres from MG63 and SAOS-2 have been investigated as many works reported the in vitro serial dissociation of sarcospheres to obtain generations expressing different tumorigenic properties². Moreover, the in vitro seeding of sarcospheres into MgHA/Coll scaffold³ has been optimized to mimic the structural configuration of the niche, where enriched-CSCs can communicate with the microenvironment in all directions, compared to standard scaffold seeding.

METHODS: The MgHA/Coll scaffolds derives from a bio-inspired biomineralization process³. The enriched-CSCs were obtained by sarcosphere-forming culture¹.

The serial sarcospheres were analysed in terms of cell proliferation (Presto Blue Reagent), Sarcosphere-forming Efficiency (SFE), Stem Cell Frequency (Limiting Dilution Test), migration and invasion ability (Transwell inserts 8 µm pores) and gene expression (qRT-PCR).

An injection-mediated seeding and the dripping of sarcospheres suspension into a pierced-scaffold were tested for the optimization of sarcospheres seeding into MgHA/Coll scaffolds, together with the possibility to close the scaffold openings by using wet material flakes. A histological analysis (Hematoxylin & Eosin), DAPI staining and, gene expression and Immunohistochemical analysis of stemness markers were performed.

RESULTS: The serial sarcospheres showed variability among generations in both cell lines. SAOS-2 sarcospheres loss the spheroidal phenotype. MG63 sarcospheres were considered and "G1" generation was selected for the optimization of sarcospheres seeding into MgHA/Coll scaffold. No relevant differences were observed between the two seeding approaches. The seeding w or w/o flakes is still work in progress.

DISCUSSION & CONCLUSIONS: Overall, the data obtained lay the basis to create a predictive in vitro 3D microenvironment of osteosarcoma.

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Keywords: Cancer, In vitro microenvironments

OP-107

Development of a hydrogel-based microfluidic model of the lymph node

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INTRODUCTION: LNs that lie immediately downstream of tumours, tumour-draining LNs (TDLNs), and directly drain factors such as proteins and metabolites, undergo profound alterations [1], [2]. These include enlargement of the LN, an increase in its cellularity, disruption in fibroblastic reticular cells (FRCs) conduits, and stiffness of the matrix. Stromal compartments within the TDLN undergo a consistent expansion and structural reorganization, and FRCs display strikingly altered transcriptional profiles. Current *in vivo* models allow little manipulation and live cell tracking, while *in vitro* models of lymph nodes are not numerous in literature and have many limitations as well. Here, we show a hydrogel-based microfluidic model of lymph node to model disease and for potential applications in drug development pipelines.

METHODS: A hydrogel-based device composed of GelMA and Collagen I was optimized with a PDMS casing to allow stable pump-driven perfusion through engineered lymphatic vessels. The new matrix blend was studied with different methods such as confocal microscopy, SEM, and second-harmonic generation microscopy. Cells in the device were retrieved at the experimental endpoint and analyzed with flow cytometry and qPCR, after cell sorting. Media from the experiment was collected and analyzed through cytokine array. The cellular macroarchitecture of the device was optimized through hydrogel extrusion and laser microdissection.

RESULTS: A new device was optimized for flow application in hydrogel and *in vivo*-like architecture was here attempted to live-track cells responding to different stimuli, general activator molecules and specific antigens. Flow cytometry was used to analyze, at the experimental endpoint, changes in gene and protein expression.

DISCUSSION & CONCLUSIONS: A hydrogel-based microfluidic model of lymph node was here created. The culture conditions and 3D environment were optimized to mimic the *in vivo* architecture and cell morphology. Cells surface markers and gene expression was analyzed and showed clear response to activation stimuli and inflammatory cues when flowed through the channels.

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Keywords: Immunity / immunomodulation / macrophage, In vitro microenvironments

OP-108

Long-lasting anastomosis on-a-chip: endogenous extracellular matrix as a key element for modelling stable and perfusable vascular network through a microfluidic device

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INTRODUCTION: One of the main issues regarding tissue engineering is represented by nutrients diffusion during the biological construct formation in vitro, due to the lack of perfusable vessel networks, which determines necrosis of millimeter-scale tissues before in vivo application. Microfluidics is one of the most increasingly used strategy due to fine control over parameters such as flow rate, velocity and shear stress, necessary to both provide nutrients and metabolites exchange at early stage of culture, and induce vascularization phenomena – angiogenesis and anastomosis – for physiological transport of molecules and biochemical signals inside a tissue.¹

METHODS: Following a previously described method for dermal micro-tissue precursors (μ TPs) production in vitro and formation of a capillary pre-network, pre-vascularized μ TPs (PV- μ TPs) were obtained with Human Umbilical Vein Endothelial Cells (HUVECs). A microfluidic device was designed with a double-channel geometry which connected two chambers in series for PV- μ TPs loading. The presence of micro-posts guaranteed PV- μ TPs confinement and generated the correct channel-chamber interface for endothelialization process. After PV- μ TPs positioning, HUVECs were seeded in the two parallel channels. A syringe pump was used to establish medium flow and guarantee oxygen and nutrient supply and metabolites release. Different time points (7, 10, 14, 18 days) of dynamic culture were analyzed to assess capillary network maturity inside the assembled PV- μ TPs. Vessels perfusability was assessed with fluorescent nanoparticles (NPs) flow.

RESULTS: Confocal and two-photon (2P) analysis demonstrated that multiple PV- μ TPs charged into each chamber of the device assembled together and formed a unique vascularized network into endogenous extracellular matrix (ECM) of tissue, stable and mature over the entire period of dynamic culture. Confocal image analysis showed an increase in vessel area fraction (AF) from day 7 to day 18, laminin production and co-localization at capillary walls. 2P excitation allowed for second harmonic generation (SHG) imaging of endogenous collagen fibers and showed collagen-vessel co-localization. Lumens were clearly visible and perfusability of capillary network was highlighted by the presence of NPs inside vessel lumens.

DISCUSSION & CONCLUSIONS: The combination of bottom-up tissue engineering approach and microfluidics represents a reliable strategy for vascularization phenomena characterization and can allow for the optimization of organ-on-chip technology including vessel networks inside an endogenous ECM. Long-lasting perfusable vessel networks can prolong shelf-life of engineered tissue

substitutes and can represent a model for physiological vascular system investigation.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Skin

OP-109

A Bone-on-Chip as a 3D in Vitro Platform to Assess the Effect of Stem Cell Age on Tissue Quality and Test Medicines

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INTRODUCTION: We present a physiologically relevant 3D environment to form human bone and disk organoids from decellularized bones and either stem cells of different ages or primary committed cells on which regenerative treatments could be tested.

METHODS: Our bone-on-chip includes decellularized bones that are recellularized with either human primary adult Mesenchymal Stem Cells (MSCs), primary and cell-line fetal human osteoblast progenitors (FHOB) or human primary annulus fibrosis (hAFC) and nucleus pulposus cells (hNPC). MSCs and FHOBs differentiated into stem cell derived osteocytes (SCDOs) within 30 days and formed bone. AFCs and NPCs formed a collagenous matrix. The systems were long-term cultures up to 26.5 months. Compressive mechanical stimulations applied to the constructs mimicked human walk.

RESULTS: Early and mature osteocytes were identified by the secretion of E11 and SOST under fluorescent confocal microscopy. Bone cells formed mineralized collagen layers of alternating orientations that created an extracellular matrix able to assemble multiple bones revealing topological cues sensed by the cells within minutes of seeding. The stiffnesses measured using 3-point bending showed primary adult MSCs formed a mineralized tissue of lower flexural stiffness than the fetal cells. FTIR measurements confirmed that Fetal cells produced highly mineralized bone that contained lipids, while adult cells assembled multiple bones to recreate the Haversian structure. AF and NP cells produced collagen Type I and II and almost no minerals.

DISCUSSION & CONCLUSIONS: The new results confirmed the influence of the age of stem cell on their ability to produce higher quality bone when from younger individuals. The bone-on-chip was also a more suitable environment for the disk cells. All systems were used as an animal-free platform to test medicines for pathologies such as HIV, cancer, and aging.

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doi.org/10.1177/02611929211025006

Keywords: Bone and bone disorders (osteoporosis etc), In vitro microenvironments

OP-110

Cardiac regeneration in a complex disease model of myocardial infarction in rats with type 2 diabetes

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INTRODUCTION: The use of pre-clinical animal models that lack the comorbid conditions present in the clinic impair their predictive power and contribute to the poor outcomes of therapies in the clinic. Here, we address the critical limitation of studies in animal models related to the lack of myocardial infarction (MI)-relevant comorbidities by studying the engraftment of human pluripotent stem cell-cardiomyocytes (hPSC-CMs) and revascularization therapy in a myocardial infarction (MI) model in rats with type 2 diabetes (T2D).

METHODS: T2D was induced by high fat diet feeding for 8 weeks and a low dose streptozotocin injection (1). After 9 weeks, when cardiac dysfunction was already present (i.e. hypertrophy and vascular rarefaction), we induced MI by performing left anterior descending artery ligation in male immunocompromised T2D rats. Cell delivery was performed by intramyocardial injection of hPSC-CMs or hPSC-CM combined with ready-made microvessels (MVs) isolated from adipose tissue (2, 3)

RESULTS: Delivery of hiPSC-CMs alone resulted in low cell survival and no differences in fractional shortening 4 weeks post-transplantation compared to hearts that did not receive any treatment. Conversely, co-delivery of hPSC-CMs with ready-made MVs promoted a 20-fold increase in hPSC-CM survival and a significant decrease in infarct area. Co-transplantation of hPSC-CM with MVs led to a significant reversal of fractional shortening loss. Pressure-volume loop analysis confirmed functional improvements in hearts that received hPSC-CM and MVs. MVs promoted a 4.6-fold increase in vessel density compared to hPSC-CM-only grafts. MVs showed long-term retention and formed the majority of the vessels in the grafts. In line with the secretion of known hypertrophic factors by MVs, (IL-6, osteopontin), hPSC-CM maturation was significantly improved specifically by MVs, as demonstrated by a 2.4-fold increase in hPSC-CM size.

DISCUSSION & CONCLUSIONS: Given the ability to use microvessels harvested from adipose tissue from patients, this study presents a new, personalized approach to cell-based therapy for heart failure post-MI whereby improving CM survival enables heart function recovery.

ACKNOWLEDGEMENTS: Work was supported by grants from the Canadian Institutes of Health Research (153160 and 180641)

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Aghazadeh et al, Cell Stem Cell (2021)

Keywords: Cardiovascular, Vascular systems / vascularisation and heart

OP-111

Perfusable vascularized stroma on-chip for growing 3D organotypic structures

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INTRODUCTION: Organoids and spheroids are increasingly recognized as key tools for "*in vitro*" studies, as they partially recapitulate the structure and function of human tissues/organs. However, there are still barriers to unlock their full potential as 3D-models. Issues such as limited oxygen/nutrients diffusion throughout the structure, often followed by growth arrest and metabolic waste accumulation, may develop necrotic cores. While this may be relevant to mimic specific developmental stages, it can also preclude tissue growth, maturation, and functional organization. To address this, we developed a microfluidic device that recreates a perfusable vascularized stromal niche for supporting spheroid and/or organoid cultures.

METHODS: The device, made by Fused Deposition Modeling 3D printing (1), had a central open liquid reservoir and the bottom was sealed with a cover slip for imaging. A needle was inserted into the device, which was filled with fibrin hydrogel with i) no cells, ii) spheroids (with fibroblasts and EC-endothelial cells, (2)), and/or iii) intestinal organoids. The needle was removed after crosslinking creating a hollow channel (300 µm diameter). This was seeded with EC under optimized conditions and connected with tubing for perfusion with a peristaltic pump. Perfusion was probed with fluorescent dextran and microbeads. Cellular organization was analyzed by immunofluorescence/confocal imaging.

RESULTS: The cell-seeding density greatly impacted the vascular channel's performance. At medium-to-high densities, EC assembled into a monolayer from which lumenized sprouts (up to 100 µm) radially formed, invading the hydrogel. This was abrogated at low cell-seeding density. The channel and sprouts could be efficiently perfused with no leakage. When spheroids were added to the system, they led to substantial fibroblast migration and formation of EC tubular structures into the surrounding hydrogel. The capillary-like structures were able to anastomose with the main channel, forming an intricate vascular network. When organoids were added, they were able to grow and mature, becoming surrounded by the perfusable vascular stroma. Notably, the whole system could be recovered from the chamber without integrity loss, opening-up a wide-range of downstream analysis options.

DISCUSSION & CONCLUSIONS: Our device combines parenchymal and stromal compartments with a perfusable vascular network. It can be adapted to the use of different spheroids and/or organoids, providing a unique "*in vitro*" platform for building biological-complexity and advancing the use of these organotypic structures as 3D-models of human tissues/organs.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Vascular systems / vascularisation and heart

OP-112

Semaphorin3A promotes the rapid assembly and long-term persistence of human SVF-derived vascular networks in engineered grafts

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INTRODUCTION: The stromal vascular fraction (SVF) of human adipose tissue is an attractive cell source for engineering pre-vascularized grafts, as it is rich in vasculogenic progenitors. We previously found that Sema3A promotes the rapid stabilization of newly formed blood vessels in skeletal muscle (Groppa E et al., EMBO Mol Med 2015). Here we investigated whether Sema3A could promote the assembly, connection to circulation and persistence of human SVF-derived microvascular networks in osteogenic grafts.

METHODS: Recombinant Sema3A was engineered with a transglutaminase substrate sequence (TG-Sema3A) to allow cross-linking into fibrin hydrogels. Grafts were prepared with 1-2x10⁶ SVF cells and hydroxyapatite granules in a fibrin hydrogel decorated with 0, 0.1, 10 or 100 µg/ml TG-Sema3A and implanted subcutaneously in nude mice.

RESULTS: After 1 week, human-derived vascular assembly was similar in all conditions. However, in the absence of Sema3A human vessels were unstable and completely regressed by 6 weeks. In contrast, low Sema3A doses (0.1 and 10 µg/ml) caused human vascular expansion by 2- to 4-fold. Similarly, the number of stromal/progenitor (non-vascular) cells was comparable in all conditions after 1 week, but again they were completely lost by 6 weeks with SVF alone. Interestingly, Sema3A preserved and further expanded seeded progenitors by 3- to 4-fold. Further, Sema3A at 10µg/ml significantly increased vascular invasion by mouse vessels already after 1 week.

DISCUSSION & CONCLUSIONS: Sema3A is a potent stimulator of the assembly and long-lasting persistence in vivo of microvascular networks derived from human SVF. Therefore, decoration of matrices with Sema3A can be envisioned to promote the rapid vascularization and functional support of tissue engineered grafts.

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Keywords: Vascular systems / vascularisation and heart, Biologics and growth factors

OP-113

Endothelial-cell sprouting assay with multiple interacting seeds as a platform to study angiogenesis

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INTRODUCTION: Spatial organization of the vasculature and interactions among microvessels play an important role in vascular network formation. The so-called bead sprouting assay based on microbeads coated with endothelial cells (ECs) and embedded in ECM-like environment is a gold standard in in vitro angiogenesis studies in 3D-microenvironments. It is based on the intrinsic ability of the ECs, forming a monolayer on the surface of the beads, to form “sprouts” which subsequently grow into complex tubular structures. In the standard assays, the “angiogenic seeds”—typically EC-coated beads or EC-spheroids—are randomly placed in the external hydrogel and the system lacks any spatial organization. A recent study (Ayan et al., 2020) has shown, however, that the distance between the seeds influences the morphology of the outgrowing vasculature. This could introduce significant bias, e.g., in angiostatic-factors screening tests. Here, we aim at developing a vascular tissue model that allows to control spatial organization of the developing vascular networks, hence overcoming current limitations. The model, based on ordered arrays of multiple co-developing and anastomosing microvasculatures, not only recapitulates key aspects of angiogenesis but also allows the development of highly homogeneous vasculatures at up to centimeter scales.

METHODS: Experimental design is based on the use of microcarrier beads coated with fluorescently labeled ECs—the model “angiogenic seeds”—embedded in ECM-like environment. We use the sprouting seeds as building blocks of larger, interconnected vascular constructs. To this end, we propose a new technique of directed-assembly of EC-coated microbeads into ordered arrays using prefabricated traps. We assemble the arrays inside a hydrogel-filled microfluidic chamber allowing long-term culture and confocal imaging of the developing microvasculature.

RESULTS: We demonstrate that we can generate arrays of highly organized architectures including square arrays of EC-coated microbeads of well defined bead-bead spacing. When embedded in an external fibrin gel the arrays evolve into a complex system of branched, interconnected capillaries. We observed that the morphology of the outgrowing vascular network strictly depends on the initial organization of the EC coated beads.

DISCUSSION & CONCLUSIONS: We demonstrate generation of ordered arrays of EC-coated beads suspended in fibrin as a platform for studying sprouting angiogenesis with possible application in high-throughput screening of anti-angiogenic therapeutics. Understanding how the spatial organization of the EC-coated beads affects angiogenic sprouting may—in the long term—help in optimizing tissue repair therapies and lead to novel biofabrication strategies such as e.g. bioprinting with angiogenic-seed enriched bioinks.

ACKNOWLEDGEMENTS: Supported by National Science Center grant Sonatina 4 (2020/36/C/NZ1/00238)

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Microenvironment and niche engineering

OP-114

Differential behaviour of mesenchymal stromal cells and pericytes in vascularization and angiogenesis in vitro

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INTRODUCTION: Vascularization of larger tissue-engineered constructs is one of the major hurdles in the field. Neovascularization can surge de novo from endothelial cell arrangement into vessels, a process is known as vasculogenesis, or develop from pre-existing vessels via sprouting angiogenesis. Mesenchymal stromal cells (MSC) are a heterogeneous mixture of cells, which can be derived from the connective tissue of different parts of the body or differentiated from induced pluripotent stem cells (iPSCs). Within this population, a subpopulation characterized by the expression of CD146 known as pericytes is the mural cells in charge of sustaining capillaries. The differential roles of MSCs and pericytes are still unclear.

METHODS: Human Umbilical Vein Endothelial Cells (HUVECs) were freshly isolated from umbilical cords. MSCs were extracted from adipose tissue, while pericytes and Human Adipose Microvascular Endothelial Cells (HAMECs) were separated from this same tissue using anti-CD146 and anti-CD31 magnetic microbeads, respectively. Induced pluripotent stem cells (iPSC) were differentiated into an MSC phenotype (iMSC)¹.

Combinations of different endothelial cell sources and supporting cell types were loaded into well-plate molded fibrin gels and culture for two weeks. The gels were then fixed with methanol, stained for CD31/PECAM-1, and observed using 2-photon laser scanning microscopy (2P-LSM).

Three-channel microfluidic chips were used to compare MSCs, pericytes, and iMSCs as supporting cell types, performing both vasculogenesis and angiogenesis assays. The chips are cultured for a week and observed in live microscopy stained with fluorescently labeled agglutinin.

RESULTS: 3D stacks of the CD31/PECAM-1 stained gels revealed a self-arrangement of the endothelial cells into capillary-like structures. HUVECs perform significantly better than HAMECs in producing a network with a higher area, volume, length, and branching points when co-cultured with MSCs. The use of CD146+ pericytes instead of MSCs significantly improves the performance of the HAMECs. Finally, the microfluidic chips show that MSCs, pericytes, and iMSCs all have the capacity to promote both the vasculogenesis and angiogenesis of HUVECs.

DISCUSSION & CONCLUSIONS: The future of tissue engineering will be marked by two of its challenges, the vascularization of thick tissues and the use of autologous cells to produce personalized implants.

This study shows that HAMECs are a feasible autologous source of endothelial cells. Furthermore, CD146+ pericytes and iMSCs showed comparable activity to MSCs in promoting both vasculogenesis and angiogenesis, proving promising autologous candidates for tissue engineering.

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from induced pluripotent stem cells." Stem cell reports vol. 3,3 (2014):414-22.
doi:10.1016/j.stemcr.2014.07.003

Keywords: Vascular systems / vascularisation and heart, Multipotent (mesenchymal) stem cells

OP-115

***In vitro* co-culture of tissue-engineered mineralised cartilage and vessel networks to model endochondral ossification**

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INTRODUCTION: Endochondral ossification (EO) is the process of bone development via a cartilage template. It involves multiple stages, including chondrogenesis, mineralization and angiogenesis. Importantly, how cartilage mineralisation affects angiogenesis during EO is not fully understood. Here we aimed to develop a new *in vitro* co-culture model to recapitulate and study the interaction between mineralised cartilage generated from human mesenchymal stromal cells (hMSCs) and microvascular networks.

METHODS: Chondrogenic hMSC pellets were generated by culture with transforming growth factor (TGF)- β 3 (10ng/ml). For mineralized pellets, β -Glycerophosphate (BGP) (10mM) was added from day 7 and TGF- β 3 was withdrawn on day 14. To evaluate the effect of the pellet secretome on angiogenesis, conditioned medium (CM) was produced by culturing the pellets for 24h in basal medium. 3D-Tube formation, transwell migration and EdU-proliferation assays were employed to evaluate the effect of the CM on human umbilical vein endothelial cells (HUVECs). To perform direct co-cultures, d14 chondrogenic or mineralised pellets were embedded in fibrin hydrogels containing vessel-forming cells (HUVECs, adipose stromal cells). The pellets were characterised by immunohistochemistry (IHC). Furthermore, the hydrogels were subjected to whole-mount staining for laminin and the vessel length density (VLD) was quantified by confocal imaging.

RESULTS: The CM from d14 chondrogenic or mineralised pellets significantly stimulated HUVEC migration and proliferation, as well as *in vitro* vascular network formation. When CM from pellets subjected to prolonged mineralisation (d28) was used, these effects were strongly reduced. When d14 chondrogenic and mineralised pellets were directly co-cultured with vessel-forming cells in fibrin hydrogels, the cartilage matrix (glycosaminoglycan and collagen type II /X stainings) and the mineral deposition (von Kossa staining) were well preserved. Confocal imaging analyses demonstrated the formation of physiologically differentiated microvascular networks, showing apicobasal polarisation as well as well-formed lumina of a regular size compatible with blood flow. Importantly, microvascular structures were formed in the proximity of chondrogenic or mineralised pellets and contact between vessels and pellets was achieved.

DISCUSSION & CONCLUSIONS: The angiogenic properties of tissue engineered cartilage are significantly reduced upon prolonged mineralisation. Based on this evidence, we developed a 3D co-culture model of vessel network formation in the presence of chondrogenic or early mineralised pellets. This model can help us to better understand the role of angiogenesis in endochondral bone formation, or find applications in disease modelling studies.

ACKNOWLEDGEMENTS: The European Union Horizon 2020 Research and Innovation Program under grant agreement 801159 supported this study.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Cartilage / joint and arthritic conditions

OP-116

Controlled metabolite release for tissue survival and integration in anoxia

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INTRODUCTION: Engineered tissues are anticipated as replacement for malfunctioning or damaged tissues and organs. However, current methods are limited to the engineering of thin tissues such as skin, or small sized tissues used in small rodent models. Scaling these tissues up towards clinical relevant sizes currently remains a major unresolved challenge in tissue engineering due to progressively severe oxygen and nutrient diffusion limitations, causing cellular necrosis and ultimately implant failures. Currently, lack of oxygen is considered the main factor leading to necrotic core formation. As a result a variety of solutions to oxygenate engineered tissues have been developed, often using peroxides to generate oxygen in situ. However, the effect of nutrient availability under diffusion limit-induced anoxic conditions has remained largely unstudied. Therefore, we have investigated the effect of various metabolites on cell survival under anoxic conditions.

METHODS: Human mesenchymal stem cells (hMSCs) were seeded, and cultured in anoxia without media change for seven days in serum-free and metabolite-free chemically defined media, except for a single type of metabolite: sugars, lipids, amino acids, or vitamins. Cell viability and functionality were determined using standard cell culture techniques. The glucose release system was engineered by thermocasting polycaprolactone-glucose into discs, which were embedded into gelatin-methacryloyl hydrogels containing 3×10^6 hMSCs/ml. Glucose release was determined using a glucose oxidase-DAB assay. Vascularization was studied using VEGF ELISA's and subcutaneous implantation in mice.

RESULTS: Sugars consistently allowed for high percentage of hMSC viability, while other conditions resulted in massive cell death. This confirms that nutrient (e.g., sugar) deprivation rather than anoxia induces cell death. Glucose was then chosen as the model sugar and was used in a metabolite release system. Here, we were able to maintain cellular viability and activity in cubic centimeter-sized engineered tissues for prolonged periods of time. Importantly, the high cell viability combined with the anoxic environment resulted in secretion of high amounts of pro-angiogenic factors, which resulted in the vascularization and therefore integration of a subcutaneously implanted tissue.

DISCUSSION & CONCLUSIONS: Metabolism supporting biomaterials are introduced as a novel method to support implant survival and realize functional integration within a receiving host. Specifically, from all metabolites, glucose is identified the key metabolite to maintain cell survival under anoxic conditions, and controlled release of glucose allows for the survival and vascularization of living implants.

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Keywords: Scaling up & manufacturing, Stem cells - general

OP-117

Scaffold-mediated delivery of small-interfering RNA to promote neuronal regrowth by silencing the expression of growth inhibitory molecules as a novel treatment for spinal cord injury

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INTRODUCTION: Following spinal cord injury (SCI), neurons lack sufficient ability drive their axons through the injury site. We propose that our biomaterial scaffold [1] made of hyaluronic acid enriched with collagen IV with optimised composition, architecture and stiffness implanted at the injury site might provide a neurotrophic bridge to support axonal regrowth and provide localised delivery of therapeutic cargoes to injured axons. Delivering siRNA cargoes to injured neurons that silence growth-inhibitory molecules can promote axonal regrowth following SCI [2]. This study thus sought to identify a suitable growth-associated pathway to target using bioinformatic methods before investigating whether our novel SCI scaffold [1] could effectively support neuronal growth and deliver siRNA to neurons to manipulate this pathway.

METHODS: First, to identify a growth-associated pathway to target, bioinformatic analyses were employed on data spanning the developmental timeframe of spinal cord neurons. Next, non-viral nanoparticles [3] were assessed for their ability to deliver siRNA to neurons. To assess the trophic ability of the scaffold to support primary culture, primary neurons were then harvested and cultured on the scaffolds. Finally, scaffolds were loaded with siRNA-nanoparticles and were assessed for their ability in delivering siRNA to neurons to manipulate the target pathway.

RESULTS: From the bioinformatic analyses undertaken, genes encoding for proteins involved in the mechanistic target of rapamycin (mTOR) signalling pathway, a master regulator of translation, were enriched in clusters of genes associated with developmentally growing neurons. Phosphatase and tensin homolog (PTEN), a potent pathway inhibitor of mTOR, was selected for non-viral siRNA targeting to enhance axonal growth. Next, a fluorescently-tagged siRNA was delivered to the cytoplasm of neurons. Subsequently, functional knockdown of the PTEN was achieved in neurons following delivery of PTEN siRNA. Building on this, a siRNA-activated scaffold delivery system was successfully established. Primary neurons cultured on scaffolds colonised the scaffold and showed robust growth of processes. Crucially, PTEN siRNA delivered from the scaffold then successfully interfered with the expression of PTEN, demonstrating potential for this system to be further developed to manipulate mTOR and promote regrowth.

DISCUSSION & CONCLUSIONS: Taken together, our results demonstrate a novel strategy to enhance the intrinsic ability of neurons to repair, ultimately developing a novel treatment for SCI.

ACKNOWLEDGEMENTS: Funding: Irish Rugby Football Union Charitable Trust and SFI Centre for Advanced Materials and BioEngineering Research

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Keywords: Gene therapy, Nervous system (brain-central-peripheral / disorders)

OP-118

Non-Viral Gene Editing Therapy for Recessive Dystrophic Epidermolysis Bullosa: CRISPR/Cas9 Mediated COL7A1 Exon 80 Excision

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INTRODUCTION: Recessive dystrophic epidermolysis bullosa (RDEB) is a rare autosomal inherited skin blistering disorder caused by mutations in the type VII collagen gene (COL7A1). Bonafont et. al. reported an electroporation based COL7A1 exon 80 deletion strategy for RDEB treatment, which efficiently restored functional type VII collagen (C7) in the RDEB patient keratinocyte cells (RDEBK) ex vivo. Following this strategy, we synthesized and tested a library of hyper branched aminated polyesters (HAPEs) as gene delivery vectors and developed topical non-viral gene editing therapies for RDEB. The CRISPR/Cas9 machinery was delivered by the selected HAPEs in either all-in-one plasmid or ribonucleoprotein complex (RNP) and achieved efficient targeted COL7A1 exon 80 deletion and C7 restoration in the RDEBK polyclones.

METHODS: A new HAPE family was synthesized, and initially screened with a plasmid encoding green fluorescent protein (GFP) on human embryonic kidney 293 (HEK) cells for DNA delivery and with Alexa Fluor 488-conjugated bovine serum albumin on HeLa cells for protein/RNP delivery. The best HAPE performers for DNA and/or protein transfection were further optimised and then formulated into complexes with (1) a plasmid encoding *S. pyogenes* Cas9 nuclease and dual single guide RNAs, and (2) a ribonucleoprotein complex, respectively, for COL7A1 exon 80 deletion. These complexes were evaluated on HEK cells and RDEBK cells, to elucidate optimal conditions for high gene editing efficiency and therapeutic effect.

RESULTS: (1) HAPE-D/DNA polyplexes achieved higher or comparable gene expression (96% GFP positive RDEBK), compared to commercial reagents including Lipofectamine™ 3000, Xfect™, jetPEI®, and jetOPTIMUS®. Furthermore, CRISPR/Cas9 plasmid delivered by the HAPE-D contributed to around 25% targeted deletion of COL7A1 exon 80 and bulk C7 restoration in RDEBK polyclones. (2) The best protein delivery HAPE (HAPE-P) demonstrated great ability on universal functional protein delivery, which was 5 times better than commercial reagents like PULsin® and Piercec™ Protein Transfection Reagent. Complexed with CRISPR Cas9 RNP, HAPE-P introduced 69% indels and 32% targeted exon 80 deletion on COL7A1 in HEK polyclones.

DISCUSSION & CONCLUSIONS: HAPEs are promising vectors for gene therapy, are easy to be modified with functional groups for different cargo delivery. The newly developed HAPEs in this study can efficiently deliver a CRISPR/Cas9 system at either DNA or ribonucleoprotein format and achieve efficient targeted COL7A1 gene deletion in vitro. The non-viral gene editing therapy developed here has a high potential for use as a topical treatment for RDEB and can be adapted to other diseases.

REFERENCES: Bonafont J, et al. Mol Ther. 2019 May 8;27(5):986-998.

Keywords: Gene editing, Wound healing

OP-119

Gene-activated surface coatings to control stem cell differentiation to osteogenic lineage

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INTRODUCTION: Bioactive compounds are a powerful tool to control cellular fate in regenerative medicine. While this research field is currently dominated by small molecules and peptides/proteins, nucleic acids move into focus. The development of delivery systems for therapeutic nucleic acids provides new opportunities to control cell behaviour. Especially local delivery systems which allow a spatially controlled action of nucleic acids are useful to develop in-situ transfection strategies avoiding systemic effects. Bone fractures represent a disorder that calls for the application of in situ transfection. Critical bone defects cause immense costs for medical treatment and reduced quality of life and will benefit from advanced therapies. Herein we present the development of gene-activated surface coatings for implants to achieve in-situ transfection of stem cells to foster their osteogenic differentiation. The functionality of the surface coating is based on biopolymers mimicking the composition of extracellular bone matrix, and lipoplexes (DNA-lipid nanoparticles) as nucleic acid delivery system encoding for bone morphogenetic protein-2 (BMP-2).

METHODS: The layer-by-layer deposition was used to construct polyelectrolyte multilayer (PEM) surface coatings composed of the polyanions chondroitin sulfate or hyaluronic acid, and the polycations collagen or chitosan. Biophysical methods were used to study the PEM formation and lipoplex embedding, including confocal microscopy, atomic force microscopy, quartz crystal microbalance, zeta potential and water contact angle measurements. Molecular biological methods were used to study the transfection efficiency and the gene transfer induced osteogenic cell differentiation.

RESULTS: PEM coatings were established with nanometer thickness possessing viscoelastic properties and moderate wettability which was also promoting cell attachment. A protocol was established to efficiently embed lipoplexes in PEMs. The functionality in gene transfer was determined using green fluorescent protein-encoding DNA which demonstrated high efficacy of transient transfection in myoblasts and mesenchymal stem cells. Stem cells growing on BMP-2 gene-activated surface coatings differentiated into the osteogenic lineage, verified by gene expression analysis and (immuno)histochemical staining of the organic and inorganic extracellular matrix

DISCUSSION & CONCLUSIONS: The developed gene-activated surface coatings represent a promising tool in regenerative medicine, allowing a spatially controllable transient genetic modification of cells. In our proof-of-concept study we demonstrate that it is possible to transfect stem cells with a gene encoding for the pro-osteogenic cytokine BMP-2. The genetically modified cells produce the cytokine which induces osteogenic differentiation due to the paracrine and autocrine mode of action. In conclusion, this strategy can be transferred on other application by exchanging the cytokine encoding DNA for localized therapeutic interventions.

Keywords: Gene therapy, Microenvironment and niche engineering

OP-120

3D-printed microRNA-loaded hydrogel scaffolds towards effective neural tissue regeneration and functional recovery following spinal cord injury

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INTRODUCTION: Spinal cord injury (SCI) can cause severe irreversible sensory and motor function loss. Inability to recover following trauma is attributed to secondary injury mechanisms that inhibit effective regeneration. While scaffold-mediated neural tissue regeneration following SCIs have shown some promising results, there is a lack of functional recovery likely attributed to the lack of remyelination in regenerated axons. MicroRNAs (miRs) are biomolecules that can regulate gene expression and modulate biological process, with miR-219 and miR-338 established as pro-myelinogenic. However, the application of miRs in biomaterial scaffolds are currently very limited, particularly in 3D-printed constructs. Here, we created miR-loaded scaffolds to assess its viability and bioactivity towards neural tissue regeneration and functional recovery following SCI. Towards this, we incorporated different biomolecules into a gelatin methacryloyl (GelMA)/polyethylene glycol diacrylate (PEGDA) bioink and formed 3D-printed hydrogel scaffolds.

METHODS: GelMA/PEGDA bioink was mixed with glial-derived neurotrophic factor (GDNF) and/or miRs (miR-219 and miR-338), 3D-printed and photo-crosslinked under visible light (405nm) to form hydrogel scaffolds containing aligned microchannels. Hydrogels were characterised, assessed *in vitro* using primary rodent neural cells, and *in vivo* in complete spinal cord transection rodent models.

RESULTS: The addition of miRs did not significantly affect the printability of the bioink or the fidelity of the printed structure. Furthermore, physical properties including swelling and degradation were not affected. miRs in GelMA/PEGDA hydrogels remained structurally intact and showed sustained release over time, as determined by gel electrophoresis and RNA quantification. When miRs released from hydrogels were transfected into myelin-producing oligodendrocyte progenitor cells (OPCs), there was an increase in myelin expression compared to the bare scaffold, as observed by immunocytochemistry and qPCR. Finally, scaffold implantation *in vivo* in complete spinal cord transection rodent models at 2 weeks showed that the additional of miRs did not adversely affect the injury site retraction and immune response. Furthermore, the combination of GDNF and miRs showed an increase in neurofilament infiltration and an increase in OPCs around the implant site compared to the bare scaffold, as assessed by immunohistochemistry.

DISCUSSION & CONCLUSIONS: Taken together, these promising results show the potential of synergistic incorporation of miRs into 3D-printed hydrogel scaffolds containing aligned microchannels towards promoting effective neural tissue regeneration following SCI. Further investigations are currently ongoing to investigate long-term tissue remodelling and remyelination *in*

vivo and subsequent functional recovery following bioactive hydrogel scaffold implantation.

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Keywords: Gene therapy, 3D printing and bioprinting

OP-121

Cystic fibrosis: lung function regeneration with non-viral gene therapy

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INTRODUCTION: Cystic fibrosis (CF) is an extremely lethal disease caused by mutations in the CFTR gene, encoding the CF transmembrane conductance regulator (CFTR) protein, and has no cure to date. CFTR gene mutations lead to abnormal chloride ion transport in epithelial tissues, which changes the hydration and pH of fluids and mucus, affecting the respiratory system [1].

Gene therapy is the most suitable approach for treating CF. In this way, gene replacement strategies have been previously developed but failed due to low gene delivery efficiency and brief expression. Although gene therapy based on viral vectors has been proven to be efficient, safety risks and immune response are important limitations [2].

In this work, we propose a different approach that consists in the development of a non-viral gene therapy based on highly branched poly(β -amino ester)s (HPAE), which family has been previously shown to perform high transfection efficiency in other genetic conditions [2].

METHODS: A family of improved HPAE polymers was synthesized and screened for plasmid DNA transfection. The selected polymers were formulated then with a range of CFTR gene-containing plasmids that were developed and improved to enhance gene expression. The formulations were evaluated in terms of CFTR protein expression and CFTR mRNA levels in CF lung epithelial cells at different time points *in vitro*. Considering these results, a formulation for inhalation was developed to be tested *in vivo*.

RESULTS: The polymer HPAE-W-A, while performing similar transfection efficiency compared to the most used commercial non-viral transfection reagents, offers better cell viability profiles. On the other hand, this polymer combined to an improved CFTR gene-containing plasmid developed, leads to the expression of CFTR protein levels up to 22.5 times the wild type human lung epithelial cells at 48 hours *in vitro*. Moreover, expression of CFTR protein was 1.3 times the non-diseased cells one week after transfection.

DISCUSSION & CONCLUSIONS: CFTR expression levels from 0.05 to 0.2 times of healthy cells are necessary to ameliorate CF patients' pulmonary symptoms [3]. The gene replacement system here developed can produce more than 100 times the CFTR protein levels necessary to achieve that goal at 48 hours in CF lung epithelial cells. Moreover, it can provide an expression superior to the healthy lung epithelial cells even 7 days after transfection, which would reduce the frequency of treatment needed, therefore facilitating its translation to the clinic.

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Keywords: Advanced therapy medicinal products, Polymers - natural / synthetic / responsive

OP-122

First step towards curing early onset genetic urinary bladder disease

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INTRODUCTION: Mutations of *HPSE2*, encoding heparanase-2, cause the autosomal recessive disease urofacial syndrome (UFS). Affected people are unable to void urine, and subsequent urosepsis causes kidney failure. *HPSE2* is expressed in bladder autonomic nerves and *Hpse2* mutant mice have aberrant bladder neuro-physiology (1-3). We hypothesized that gene replacement therapy would cure these mice.

METHODS: AAV9-CAG/*HPSE2* was administered to neonatal mice by intravenous injection. Two to three weeks later, transgene transduction and expression was assessed by Basescope *in situ* hybridization on histology, and *ex vivo* physiology experiments were undertaken to assess therapeutic efficacy.

RESULTS: We demonstrated that the transgene was detected in, and expressed by, bladder ganglia, structures that innervate the bladder, with a lower level of expression in the bladder itself. Aberrant physiology of the bladder body and also the outflow tract was ameliorated in *Hpse2* homozygous mutant mice that had been administered AAV9 as neonates.

DISCUSSION & CONCLUSIONS: This study is a first step towards curing UFS, a clinically devastating early onset Mendelian urinary bladder disease.

ACKNOWLEDGEMENTS: MRC, Kidneys for Life and Kidney Research UK

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2. Roberts *et al Kidney Int* 2019 PMID: 30885509

3. Manak *et al NeuroUrol Urodyn* 2020 PMID: 32609936

Keywords: Disease models, Nervous system (brain-central-peripheral / disorders)

OP-123

The development of non-viral gene editing strategies to permanently correct ATM loss in the brain for A-T therapy

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INTRODUCTION: Ataxia-Telangiectasia (A-T) is an autosomal recessive disorder that results AT-mutated (ATM) gene mutations with those affected having phenotypical hallmarks of ataxia, which results from neurodegeneration of Purkinje cells in the cerebellum. Prognosis for patients is incredibly poor, being wheelchair-bound in teenage years and lethal by thirties. The current approved treatments for A-T only work at a symptomatic level; none slow or stop the loss of neurons, therefore providing a therapeutic avenue to halt or reverse neurodegeneration is needed.

METHODS: In developing an efficient strategy for non-viral nucleic acid therapy for A-T, we have focused on improving an already efficacious system which utilises glycosaminoglycan-binding enhanced transduction (GET) technology for non-viral gene delivery of nanoparticles (NPs).

RESULTS: We have demonstrated that GET-mediated delivery of plasmid (p)DNA encoding ATM as GET-NPs in ATM-/- cells can effectively rescue the irradiation sensitive phenotype of AT and that GET-ATM delivery is possible in in-vivo to mice cerebellum. Our current work aims to enhance penetration of the GET NPs for wide-spread cerebellum distribution, retaining high Purkinje cell transfection levels and promoting long-term therapeutic activity.

DISCUSSION & CONCLUSIONS: We are testing several strategies to convert transient ATM expression to a stable, genome correction of A-T by gene editing technologies which will further be tested for efficacy and safety in brain delivery of experimental animals. Overall, this research provides a novel exciting avenue which has the potential to pave the way for future non-viral gene editing therapeutics for A-T and other brain diseases.

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Keywords: Gene editing, Nervous system (brain-central-peripheral / disorders)

OP-124

Influence of long-term storage of cell-laden alginate-methylcellulose based bioinks on printability as well as cell viability and function

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INTRODUCTION: Bioprinting is a promising tool on the way to make space missions less dependent on medical care on Earth. However, preparing bioinks consisting of suitable biomaterials and living cells is a complex process that could be done a lot easier on ground than in microgravity. However, a long storage time of the prepared inks is required when sending them into space. In the present study, we investigate the influence of a storage of up to 28 days at 4°C on printability, cell viability and functionality of an alginate-methylcellulose based bioink.

METHODS: The microalgae strain *Chlorella vulgaris*, an immortalised human mesenchymal stem cell line (hTERT-MSC), the human osteosarcoma cell line SAOS-2, the human hepatocellular carcinoma cell line HepG2 and primary human dental pulp stem cells (DPSC) of two donors were investigated. Alginate-methylcellulose based biomaterial inks were prepared in water (Lode 2015), PBS (Schuetz 2017) or human fresh frozen plasma (Ahlfeld 2020), depending on the cell type. At different time points of storage, rheological properties were determined and 3D bioprinting was performed using a Bioscaffolder 3.1 from GeSiM (Germany). Bioprinted constructs were cultivated up to 28 days and embedded cells were analysed for their viability via fluorescence microscopy after live/dead staining and cell number development through chlorophyll extraction (algae) or DNA quantification.

RESULTS: All compositions showed good printability even after storage times of up to 28 days and the scaffolds displayed good shape fidelity. *Chlorella vulgaris* showed no loss of viability, good cell growth and no significant negative influence on the photosynthetic efficiency related to the different storage times. For all mammalian cell types, storage itself but not storage duration was shown to affect cell viability. In almost all cases, the viability recovered over the course of cultivation.

DISCUSSION & CONCLUSIONS: The data indicate that pre-mixing of alginate-methylcellulose bioinks prior to their transport to space might be possible: Storage at 4°C for 28 days, considered to be a realistic timeframe for a launch to the ISS and subsequent use at the space station, did not hamper 3D bioprinting. Furthermore, it is clearly evident that the studied conditions are suitable for *Chlorella vulgaris*. For all mammalian cell types, the viability results are promising, but clearly indicate that the method needs to be further optimised.

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Keywords: 3D printing and bioprinting, Biomaterials

OP-125

Diffusion Packing: The mechanism behind embedded 3D printing of dilute cell and particle suspensions into dense tissue structures

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INTRODUCTION: Embedded 3D printing enabled an unprecedented degree of control over the print design and materials that can be used as inks. In order to fabricate functional microtissues, a high cell density is usually required. However the maximum cell concentration that can be incorporated in a hydrogel ink before the ink's rheological properties are compromised, is significantly lower than the concentration in a tissue equivalent. Extruding dense cell suspensions, generally results in high shear stresses on the cells and a poor shape fidelity of the print. In this work we report the previously unstudied mechanism of diffusion packing, that occurs when dilute particle suspensions in Newtonian fluids are printed within shear thinning embedding baths, resulting in complex, dense tissue structures.

METHODS: In order to visualize the diffusion packing process a custom imaging set-up was prepared which allowed real time multicolor fluorescent imaging while printing. We compared shear thinning baths and newtonian fluid baths, using a variety of flow rates and printing speeds. We used density matched particle suspensions comprising of Polystyrene, Iron oxide (III) solid particles, Alginate and collagen coated PLGA spherical microbeads, Smooth Muscle Cells an spheroids, Induced Pluripotent Stem Cells, Cardiomyocytes, Epicardial cells, and combinations of the aforementioned.

RESULTS: We visually verified that diffusive packing occurs when a dilute particle suspension in a Newtonian fluid carrier is printed within a shear thinning bath. By testing different flow rates, nozzle sizes we established an operating window where diffusion packing allows the dilute particle suspension to pack into a very dense particle feature. Controllable meshes and sheets up to 6x6 cm were also printed consisting of particles. The printed feature diameter ranged from 100µm to 2mm, and was largely independent of the nozzle diameter, but relied in the particle fraction of the ink suspensions and the infill value. Suspensions that combined Collagen coated particles and Smooth Muscle Cell spheroids and cells formed composite tissues. We demonstrated the self-organization of functional cardiac tissue fibers with a layer of epicardial cells surrounding a body of cardiomyocytes.

DISCUSSION & CONCLUSIONS: We studied the printing of dilute suspensions in shear thinning baths. The mechanism of diffusive packing allows the creation of dense 3D printed features in shear thinning embedding baths, without the bath material intermixing with the particles. This mechanism was verified using a variety of suspensions, ranging from solid Iron oxide(III) particles, to cell spheroids. Diffusion packing has definitely occurred in previous published works, but had not been studied.

Keywords: 3D printing and bioprinting, Induced pluripotent stem cells

OP-126

A 7 Degree of Freedom robot for volumetric biomimetic scaffold fabrication and tissue engineering

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INTRODUCTION: Most of the current additive manufacturing (AM) technologies that are used for biofabrication and tissue engineering (TE) work in a layer-by-layer approach, limiting the architectural complexity of the constructs that can be produced. For instance, it is very difficult to obtain scaffolds with anisotropic fiber orientation in 3D through extrusion based AM technologies. A motion system with higher degrees of freedom (DOF) could be used for volumetric extrusion-based 3D printing. Indeed, there have been a few successful attempts in developing robotic AM platforms both from academia and industry, but we are currently lacking such an approach on the small scale that is needed for TE applications.

METHODS: A new custom-made robotic AM platform was developed integrating a standard fused filament fabrication (FFF) extruder and a 7DOF robot (Sawyer robotic arm, Rethink Robotics). A precise control on motion and extrusion was achieved through the development of a custom code, which was based on the open-source Robotic Operating System (ROS) and allowed to synchronously send signals to the extruder during the motion of the robot. The planning and execution were done for each segment based on a text file containing the printing parameters and the trajectory, provided as Cartesian points, starting from a custom-made Gcode for AM control. The motion planning process and accuracy of motion were optimized by using different open source planning algorithms and changing some relevant parameters.

RESULTS: The new platform can easily print nonplanar paths, giving it the ability to create even more intricate scaffolds that better mimic the architecture of native tissues. The development of a custom-made slicing platform allowed to print nonplanar geometries on nonplanar surface geometries (e.g., tilted printing beds), thus paving the way towards *in vivo* bioprinting.

DISCUSSION & CONCLUSIONS: This new AM platform allows for extra capabilities to the system, such as collision avoidance and smoother motions in 3D. This will also make it possible to vary the orientation of the extruder during motion, thus acting on the fibers alignment. Furthermore, such system will eventually allow for volumetric printing by integrating advanced cooling systems. In short, this new platform opens up vast opportunities in small scale AM specifically for regenerative medicine.

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Keywords: Additive manufacturing, Biofabrication

OP-127

optimise hydrogels for bioprinting with machine learning

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INTRODUCTION: This (cartilage) hydrogel mimics the principal components of the chondrocyte's extracellular matrix¹, glycosaminoglycans and collagen, using chondroitin sulphate (CS) and gelatine (GEL), respectively, with 4-arm polyethylene glycol (PEG) as a spacer agent providing structural support. The three polymers are chemically crosslinked. A Machine learning algorithm is under development to predict the mechanical and rheological properties of potential materials based on varied inputs such as the concentration of polymer precursors. The algorithm should also suggest potential formulations, which can be processed and validated through printing experiments.

METHODS: 27 different formulations of hydrogel were tested with:

-Mechanical testing: compression under 70% strain.

-Rheology: 1) flow ramp at 37°C under a wide range of shear rates (0.01-1000s⁻¹); 2) time sweep followed by a temp sweep with settings at 37°C fixed strain 1% and 1Hz for 2h, 25-90°C heating rate 5 °C/min respectively.

Results from the tests are fed into a supervised machine learning algorithm, Using the final trained model, we aim to identify the best parameters for which the model predicts ideal and desirable rheological and mechanical properties. Formulation of this new hydrogel will allow validation of the algorithm's prediction accuracy.

RESULTS: Early results on the rheological characterisation show increasing the concentration of GEL resulted in higher viscosity at given shear rates. This trend was also seen in the increase in EDC/NHS. All materials are shear-thinning. However, their viscosity is too high compared to the literature², implying these formulations might not be processable using 0.25 mm nozzles. An increase in nozzle size could prevent clogging but would sacrifice the printing resolution. Therefore, the formulation must be optimised.

Preliminary results from compression testing show that the hydrogel's Young's Modulus can range from 30 to 200 kPa, which is within the desired range for cartilage applications, 100kPa and higher¹.

DISCUSSION & CONCLUSIONS: A biomimetic and bioprintable hydrogel is formulated with potential for biocompatibility and chondrogenesis, due to the use of polymers found in the ECM of cartilage and high modulus. A new method of synthesising hydrogels is currently under development using machine learning, with the aim to improve efficiency, and saving costs by reducing material waste. The method is customisable and easily adaptable for the use case of the researcher.

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Keywords: 3D printing and bioprinting, Hydrogels and injectable systems

OP-128

Aqueous Two-Phase Enabled Low Viscosity 3D (LoV3D) Bioprinting of Living Matter

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INTRODUCTION: Embedded bioprinting is a promising additive manufacturing technique permitting fabrication of large-scale, freeform, complex 3D tissue constructs. During the development of new (bio)inks, the shape-stability of extruded strands plays a major role. To stabilize strands post-extrusion, while allowing for smooth extrusion with limited pressures, shear thinning inks are commonly employed. For these inks, printing resolution is dictated by nozzle diameter[1], while shear stresses within the nozzle during extrusion determine cell viability[2], which limits bioprinting resolution and speed. Here, we report on a low viscosity 3D (LoV3D) liquid printing approach, which is enabled by the innovative use of an aqueous-two phase system (ATPS). LoV3D allows for highly rapid bioprinting with high cell viability, while allowing for continuous on-the-fly tuning of filament diameter.

METHODS: For 3D printing of several inks, a Cellink Inkredible+ printer was combined with a syringe pump to allow flow rate and print speed control. Various mammalian cell types including 3T3 mouse fibroblasts were used to investigate LoV3D's cytocompatibility.

RESULTS: The suitability of a variety of established (bio)polymer solutions for LoV3D printing was confirmed and Dextran TA/PEG as well as Alginate TA/PEG were chosen as model systems. The formation of a stable ATPS interface was substantiated by establishing the binodal curves of these systems. The use of low viscous solutions allowed for strand elongation (thinning) with increased printing speeds down to single-cell width, offering a substantially higher resolution printing regime compared to literature. The influence of viscosity dependent shear stresses within the printing nozzle on cell viability was investigated by combining simulation-based data with extrusion experiments. Here, we were able to establish that low viscosity inks do not show significant reduction of cell viability, while higher viscosity inks reduced the viability with increasing flow rates down to 70%. Complex shapes such as spirals, tubes, and grids could readily be created. Incorporating cells within a sacrificial ink allowed for a one-step print and seed approach.

DISCUSSION & CONCLUSIONS: In this study, we introduce LoV3D bioprinting as a highly versatile and rapid bioprinting technique, which is inherently compatible with a wide range of biomaterials and crosslinking strategies. Future studies will focus on cell incorporation within the channels, aiming for the creation of perfusable multiscale vascular networks.

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Keywords: 3D printing and bioprinting, Biofabrication

OP-129

Guiding cells with light: Patterning of cell-laden hydrogels using multi-photon lithography

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INTRODUCTION: Hydrogels can mimic the extracellular matrix (ECM) and provide a suitable environment for 3D cell culture [1]. Multi-photon lithography (MPL) is a versatile tool that can be used to initiate photochemical reactions at a micrometer resolution. We utilize this method to alter the chemical and mechanical properties of cell-laden hydrogels and guide cell migration.

METHODS: Gelatin methacryloyl (Gel-MA), supplemented with the photoinitiator (PI) (2,4,6-trimethylbentoyl) phenylphosphinate (Li-TPO), is crosslinked using a UV curing chamber. After crosslinking, it is soaked in a 1 mM 4,4'-diazido-2,2'-stilbenedisulfonic acid (DSSA) solution. Gelatin norbornene (Gel-NB) is supplemented with dithiothreitol (DTT) as a crosslinker and a modified diazosulfonate-based (DAS) photoinitiator at an equimolar thiol-ene ratio. A custom-built, as well as a commercial (NanoOne Bio, UpNano GmbH) MPL systems, were used to pattern the hydrogels. Three different cell types are used in the present study, namely human adipose-derived mesenchymal stem cells (hASCs/TERT1, Evercyte), human umbilical vein endothelial cells (HUVECs, PELOBiotech) and L929 fibroblast cells.

RESULTS: In the first method, a hydrophilic molecule (DSSA) is covalently bound to the C-H groups of Gel-MA in a process called photografting [2]. By creating a 3D pattern with varying laser power directly around the cell spheroids, we were able to show that hASCs are preferentially migrating into regions that have been exposed to a higher laser dose. DSSA-free samples that were exposed to the laser showed no preferential migration direction. This indicates that a higher laser power leads to an increase in the density of bound DSSA molecules and that cells prefer to migrate into regions with a higher DSSA density. In the second method, Gel-NB is crosslinked upon initial laser irradiation and amide bonds of the gelatin backbone are cleaved following a subsequent laser exposure [3]. The combination of a highly efficient PI with an on-the-fly optical voxel tuning facilitates the fabrication of centimeter large structures with a micrometer resolution. A perfusable spheroid-containing hydrogel structure is printed inside of a microfluidic chip, 3D patterns are cleaved around the spheroid and the migration of L929 cells is observed.

DISCUSSION & CONCLUSIONS: We have demonstrated two different approaches to altering the chemical and mechanical characteristics of cell-laden hydrogels using MPL. Both approaches satisfy the requirements of biocompatibility, high-resolution and high complexity that are necessary to manipulate the microenvironment of cells.

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Keywords: Biofabrication, In vitro microenvironments

OP-130

Enabling strategies for printing human photopolymerizable blood components with low viscosity and high fluidity

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INTRODUCTION: Human blood-derived platelet lysates (PL) have recently arisen as a source of highly biocompatible xeno-free biomaterials with intrinsic cell-recruiting and pro-regenerative capacity. Albumin represents 41% of the PL composition, thus BSA (bovine serum albumin) can be used as a cheaper and standardized alternative for optimizing the processing involving a photo-cured printing system. Due to the poor stability and unsuitable rheological properties of PL and BSA inks, it is not possible to print them using standard printing techniques. Hence, the objective of this work was focused on enabling strategies for obtaining printed PL- and BSA-based scaffolds with satisfactory printing shape, fidelity, and stability.

METHODS: Methacrylated PL and BSA inks were synthesized to obtain photopolymerizable materials. First, their use in combination with the natural polysaccharide xanthan gum (XG), using a double ionic/photo crosslinking approach with iron ions was explored. Then, their use without any other external thickener by the freeform embedding in suspended hydrogels (FRESH technique), photocrosslinkable using Ruthenium, was implemented. The ideal suspended hydrogel was obtained after optimizations of pH, temperature, and polymer ratio, using a complex coacervation process.

RESULTS: In both strategies, excellent printing performance and satisfactory shape fidelity were obtained. In combination with XG, the range of fibers obtained was 100-200 µm. With the FRESH technique, fibers with 250-580 µm, and 500-µm diameter rings were also manufactured, depending on the characteristics of the supporting hydrogel, especially hydrophilicity. High cell viability using mesenchymal stem cells was also observed in both strategies. In combination with XG, higher stability in culture medium for 28 days and improved mechanical properties were observed. Alternatively, with the improved FRESH technique here developed, xeno-free constructs were obtained and a controlled release of growth factors and proteins over 50 days could be observed. These highly bioactive printed scaffolds obtained with both strategies were combined in a multi-material approach with polycaprolactone-poly(lactic acid) (PCL-LA copolymer) meshes, produced by melt-electrowriting (MEW). The range of fibers obtained by MEW was 30-50 µm and the interfiber space was 0.5-1 mm.

DISCUSSION & CONCLUSIONS: Based on the use of both strategies, satisfactory printed PL and BSA scaffolds were obtained, which after proper hybridization between MEW and bioprinting resulted in promising multi-material scaffolds. These hybrid scaffolds could find applications for the regeneration of multi-tissue constructs such as in case of the temporomandibular joint.

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Keywords: 3D printing and bioprinting, Enabling technologies

OP-131

Protein-based hydrogel tissue adhesives for failing hearts

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INTRODUCTION: Ischaemic heart disease leading to heart failure is the principal cause of morbidity and mortality globally [1]. Lack of blood flow to the myocardium leads to cardiomyocytes death, fibrotic remodelling in contractility impairment and loss of functions [2]. Exosomes (small extracellular vesicles) offer new hope to address this unmet clinical need, but their success is still hampered by biodistribution and delivery issues. Here, we propose biocompatible, protein-based hydrogels as tissue adhesive regenerative heart patches to locally release therapeutic exosomes.

METHODS: We chemically modified proteins and characterised the degree of functionalisation via ¹H NMR spectroscopy. Protein-based hydrogels were prepared by irradiation of the modified proteins in the presence of a photoinitiator. Different hydrogel formulations were designed by changing protein (10, 15, 20% w/v) and photoinitiator concentration (low and high) to identify the most promising biomaterial candidates. Crosslinking kinetics were investigated via rheometry; mechanical properties via compression tests; stretchability via tension tests; tissue adhesion to a collagen sheet model via pull-off tests. We engineered human mesenchymal stem/stromal cells (MSCs) to produce GFP-labelled exosomes and encapsulated them inside the hydrogels. Cell viability and morphology was evaluated via resazurin assay and live/dead staining. Hydrogel adhesion to the epicardium (external layer) of the beating left ventricle was assessed ex-vivo by using freshly isolated mouse hearts.

RESULTS: We successfully modified proteins as evidenced by the appearance of characteristic peaks in the NMR spectra. Protein-based hydrogels successfully crosslink upon irradiation, as shown by increased rheological properties after exposure to light. The rheological properties (storage modulus=0.4-40 kPa) and mechanical properties (elastic modulus=1-18 kPa) were tuned by varying design parameters (lower for lower protein and photoinitiator concentration). Hydrogels were characterised by stress and strain at failure under tension of 2-8 kPa and 20-150%, respectively. All hydrogels adhered to collagen sheets, with adhesion strength (2-20 kPa) increasing with higher photoinitiator concentration. Viable MSCs were distributed in 3D within the hydrogels and their metabolic activity increased over time. Cell elongation was guided and achieved by tuning the physico-mechanical properties of the hydrogels. Hydrogels successfully adhered to beating hearts.

DISCUSSION & CONCLUSIONS: We synthesised and tuned the properties of protein-based hydrogels and selected promising candidates as heart adhesives [3]. We are currently investigating exosome release from MSC-laden and exosome-laden hydrogels.

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Keywords: Hydrogels and injectable systems, Cardiovascular

OP-132

Rapamycin prevents early post operative failure of Tissue Engineered Vascular Grafts

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INTRODUCTION: As previously reported our Tissue Engineered Vascular Grafts (TEVG) implanted in a large animal model have shown early post operative narrowing (stenosis) on catheter angiography and Intra Vascular Ultrasound (IVUS). Stenotic grafts often have a need for re-interventions due to low cardiac output, fluid accumulation and other complications. To address these, we developed a TEVG conjugated with Rapamycin (rTEVG).

Rapamycin is an antiproliferative drug that reduces stent stenosis and pulmonary vein stenosis. Our study objective is to evaluate rTEVG safety and efficacy at preventing early post operative stenosis and long term tissue regeneration.

METHODS: We conjugated Rapamycin to poly glycolic acid felt infused with a copolymer of poly-ε-caprolactone and poly-L-lactide. rTEVGs (16mm, 7mg/cm², n=4 and 35mg/cm², n=4) and additional control TEVG were implanted in the sheep thoracic inferior vena cava. Previously reported results from our lab for control TEVGs were utilized as a control data (n=8) for stenosis (%) (mid-graft narrowest diameter/1wk mid-graft diameter), stenotic graft area (%) (mid-graft narrowest area/1wk mid-graft area) were evaluated with angiography and IVUS at 1, 8, 12, 26, and 52 weeks. Subsequently, animals were sacrificed rTEVG n=2, non-rTEVG n=1 at 8 week, and rTEVG at 1 year n=4 for histological evaluations.

RESULTS: There was one graft related animal death in control graft group but no deaths in rTEVG groups (P=0.32). Compared to control TEVG, rTEVGs were significantly less stenotic at the 8-wk time point as measured by both of angiography (control vs 7mg/cm²; 55.4 vs 28.8%, P=.001, control vs 35mg/cm²; 55.4 vs 38.3%, P=.017) and IVUS (control vs 7mg/cm²; 68.1 vs 33.9%, P<.001, control vs 35mg/cm²; 68.1 vs 45.8%, P=.01). One sheep with a 7mg/cm² rTEVG, one control TEVG were sacrificed for 8-wk histological evaluation showed much less inflammatory and smooth muscle cell (SMC) migration into both the intra-scaffold and neointimal layers of rTEVG than the control TEVG.

To data, 4 rTEVGs (35mg/cm², n=3 and 7mg/cm² n=1) have reached 1-year evaluation. The 7mg/cm² rTEVG showed consistent mechanical properties as well as appropriate regeneration to native tissue same as control TEVG. 35mg/cm² rTEVGs showed significant stenosis with intimal dissection indicating regeneration was impaired.

DISCUSSION & CONCLUSIONS: Rapamycin may prevent early post-operative TEVG stenosis. However, too much Rapamycin delayed tissue regeneration and did not prevent long-term TEVG stenosis. In this study, 7mg/cm² Rapamycin prevented early TEVG stenosis by decreasing inflammatory and SMC migration, while allowing for long-term tissue regeneration.

Keywords: In vivo and animal models, Vascular systems / vascularisation and heart

OP-133

Unveiling the function of the mechanosensor YAP in the activation of cardiac fibroblasts

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INTRODUCTION: Cardiac fibrosis, one of the primary reasons of end-stage heart failure, is a pathological process mediated by cardiac fibroblasts (cFbs). It is determined by the maladaptive remodeling of the heart extracellular matrix (ECM), resulting in its excessive deposition and stiffening. CFbs can be activated by mechanical stress created by this remodelling, which makes Yes-associated protein (YAP), a mechanosensor, a mediator of this process. Given the lack of therapies against fibrosis, Yap provides a possible target for future treatments.

METHODS: Human PSCs depleted or not from YAP were differentiated into cFbs (YAP ^{-/-} and WT cFbs) using a published method [1]. The resulting cFbs were characterized by immunofluorescence and flow cytometry (FC). To evaluate their functionality, the collagen gel contraction assay was used to assess ECM remodeling. Also, YAP WT and ^{-/-} cFbs were exposed to fibrotic or non-fibrotic stimuli for 5 days to study how they transdifferentiate into myofibroblasts through the quantification of α -SMA and FAP expression by FC and SMAD2/3 nuclear localization by IF. RNA sequencing (RNASeq), of primary human cardiac fibroblasts (HCF) and normal human dermal fibroblasts (NHDF), together with YAP WT and ^{-/-} cFbs, was performed to disclose the genetic landscape driving cFb differentiation

RESULTS: YAP ^{-/-} PSCs showed an impaired capability to differentiate into cFbs demonstrated by the lower expression of cardiac markers (NKX2.5 and GATA4) compared to YAP WT cFbs. This was confirmed by RNASeq which revealed more similar profiles between YAP WT cFbs and HCF than the YAP ^{-/-} cFbs. Also, the latter show impaired functionality demonstrated by their hindered capability to contract collagen gels compared to the WT cFbs. These cells also show disrupted capability to transdifferentiate as shown by the erratic expression of α -SMA and FAP and lower number of nuclear SMAD2/3+ cells.

DISCUSSION & CONCLUSIONS: Our results show that YAP has a role in cFbs differentiation and functionality by downregulating the expression of cardiac markers, impairing ECM remodeling capabilities and disrupting transdifferentiation into myofibroblasts. RNASeq using HCF and NHDF provided a genetic fingerprint allowing us to obtain a list of possible YAP targets that could be involved in driving cFbs differentiation. These results will be complemented with chromatin immunoprecipitation sequencing to provide better specificity.

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Keywords: Cardiovascular, Biomechanics / biophysical stimuli and mechanotransduction

OP-134

Engineering of Bacterial Cellulose-Based (BC) Vascular Graft for Coronary Artery Bypass Grafting (CABG)

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INTRODUCTION: Coronary artery disease is one of the leading causes of death worldwide¹. In the context of coronary artery bypass grafting (CABG), it is not always possible to use autologous substitutes². Synthetic vascular grafts (e.g., Dacron (PET), Teflon (ePTFE)) are an alternative option only for substitution of vessels with an inner diameter >6mm^{3,4} and not suitable for CABG. The aim of the project is to develop a Bacterial Cellulose-based (BC) small diameter (4mm) vascular graft for CABG. BC is a natural biomaterial synthesized extracellularly by bacteria. It is characterized by great biocompatibility and excellent mechanical properties. In a proof-of-concept study, first prototypes of BC grafts were used for CABG in pigs. After 4 weeks, angiography showed fully open grafts with no dissection and no narrowing at distal anastomoses. Histology of the explants showed endothelialization from autologous endothelial cells and formation of neointima⁵ over the entire length of the graft. The second-generation BC grafts were produced and tested biomechanically. The hypothesis of the study is that BC grafts are biomechanically suitable to substitute autologous saphenous veins.

METHODS: BC grafts are generated in a customized bioreactor providing a temperature- and oxygen-controlled culture environment by *Gluconacetobacter*.

RESULTS: We manufactured up to 15cm long BC grafts with a 4mm inner diameter (wall thickness 0.49 ± 0.04 mm, n=6), a circumferential tensile strength of 2.32 ± 1.12 MPa (force at the breaking point of 1.85 ± 0.82 N, n=4), a burst pressure strength up to 367.51 ± 23.09 mmHg (n=3) and a suture retention strength of 0.72 ± 0.33 MPa (n=3). BC grafts properties are compared with the human saphenous vein (SV). At the morphological level BC grafts and SV showed comparable wall thickness (SV: 0.55 ± 0.13 mm, n=15). However, SV were characterized by side branches sealed by metallic clips. Under the mechanical point of view, SV shown a burst pressure strength up to $718.7.7 \pm 669.8$ mmHg (n=10) and a circumferential tensile strength of 1.76 ± 1.04 MPa (n=5).

DISCUSSION & CONCLUSIONS: Autologous grafts showed in general a high inter- and intra-graft variability in terms of mechanical properties compared to BC grafts. This may be due to the branching points or to the actual quality of the patient blood vessel. Although SV burst pressure strength was higher than BC, BC values were still almost the double of physiological blood pressure. These data indicate the engineered BC graft could be a promising alternative to autologous grafting for CABG procedure thanks to their homogeneous quality and mechanical performance.

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Keywords: Cardiovascular, Vascular systems / vascularisation and heart

OP-135

RNA binding protein hnRNPC is a mechanosensitive component of RNA homeostasis apparatus in the failing heart

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INTRODUCTION: The onset and progression of aging-associated pathologies is paralleled by continuous local extracellular matrix (ECM) remodelling, which serves as a compensatory strategy for the tissue to cope with altered conditions (1).

Cardiac pathologies are a paradigmatic example of how negative ECM remodelling eventually leads to heart failure. Cardiomyocytes respond to the ensuing biomechanical stress by re-expressing fetal contractile proteins via transcriptional and posttranscriptional processes, such as alternative splicing (AS).

METHODS: We adopted pluripotent stem cells, bioengineering tools, molecular biology and bioinformatics to dissect the role of heterogeneous nuclear ribonucleoprotein C (hnRNPC) - an RNA-binding protein (RBP) mainly involved in mRNA splicing and maturation in the adult cardiomyocytes - in human failing heart.

RESULTS: First, we demonstrate that ischemic and chronic cardiac pathologies are accompanied by the altered expression of the RBP heterogeneous nuclear ribonucleoprotein C (hnRNPC). By using super-resolution microscopy, RNA-sequencing and mass spectrometry, we demonstrate hnRNPC relocates to the sarcomeric Z-disc upon ECM pathological remodelling, where it associates with active sites of localized translation.

We additionally prove that alterations in hnRNPC expression, phosphorylation, and localization can be mechanically determined and affect the alternative splicing of mRNAs involved in mechanotransduction and cardiovascular diseases, including Hippo pathway effector Yes-associated protein 1 (YAP1). In particular, we found that the mechanically induced hnRNPC displacement or its depletion affect the inclusion of YAP1 exon 4. This splicing event is of particular relevance, since the exon encodes for the second WW domain, a domain known to directly control the protein interaction with TEAD transcription factor and thus its DNA binding activity. This switch towards the expression of YAP1 isoforms with two WW domains (YAP1-2) has been recently described by our research group in human pathological hearts (2, 3).

DISCUSSION & CONCLUSIONS: In conclusion, our results on the regulation of hnRNPC localization and function in diseased myocardium unveil a novel layer of mechanosensing through which RNA homeostasis could undergo mechanical regulation following pathological cardiac ECM remodelling.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Cardiovascular

OP-136

Reactive jet impingement (REJI) 3D bioprinting for cardiac tissue cultures

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INTRODUCTION: REJI 3D bioprinting for cell-filled gels was developed in Newcastle University. REJI utilizes high-speed jetting of cell-loaded bioactive materials to form droplet streams that collide mid-air before they rapidly cross-link and deposited onto a substrate on a drop-on-demand basis. The key advantage of the technology lies on the high cell densities (up to 40M-50M/mL), the diversity of substrates it can print on and the minimized cell damage compared to syringe-based methods. It can be used to create overconfluent cell-loaded gel structures that produce higher quality tissues in a shorter period.

METHODS: HL-1 cardiomyocytes were printed Collagen-Alginate-Fibrinogen (CAF) hydrogel 3D structures, cross-linked by a cell-loaded Thrombin-CaCl₂ ink. HL-1 cells were selected due to their capability to spontaneously contract and be serially passaged, while maintaining their cardiac phenotype. The 3D cultures were printed at 10-20M/mL directly into 24-well plates and cultured for 7 days.

RESULTS: The cells demonstrated high confluency from day 1 and presence of myosin and desmin proteins involved in cardiac muscle function from day 3. Contractility due to spontaneous calcium fluctuations associated with the presence of voltage dependent currents was maintained throughout the study and was visualized by a calcium flux assay.

DISCUSSION & CONCLUSIONS: The results confirm the viability of HL-1 cells in CAF hydrogel and serve as a basis for further development of this work to the bioprinting of contractile cardiac tissue.

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Keywords: Additive manufacturing, Hydrogels and injectable systems

OP-137

Electroactive microenvironments for cardiac tissue regeneration

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INTRODUCTION: As an electrically and contractile tissue, cardiac tissue would benefit greatly from the use of a scaffold that is electrically conductive and capable of promoting cellular activity, and mimicking the mechanical and structural properties of the native cardiac extracellular matrix. In this regard, electroactive scaffolds are increasingly being used in cardiac tissue regeneration strategies, alone or in combination with existing materials, namely conductive ones, to actively recreate tissue microenvironment. This work reports on the novel combination of poly(vinylidene fluoride-co-trifluoroethylene) (P(VDF-TrFE)) with ionic liquids (ILs), 2-hydroxyethyl-trimethylammonium dihydrogen phosphate ([Ch][DHP]) and choline bis(trifluoromethylsulfonyl)imide ([Ch][TFSI]), or with graphene oxide (GO) to achieve scaffolds with improved properties for cardiac tissue regeneration. Poly(vinylidene fluoride) (PVDF) membranes with different surface charges (poled and non-poled) were also used to investigate their influence on H9c2 cell adhesion and proliferation under static and dynamic (mechano-electrical stimulation) conditions. **METHODS:** P(VDF-TrFE) films were reinforced with GO and with ILs up to 20% wt. of filler content. The morphological, physico-chemical and thermal properties of the composites were analysed, as well as their potential use as scaffolds for cardiac repair. H9c2 cell adhesion and proliferation on the different PVDF samples were assessed by immunocytochemistry and resazurin assay.

RESULTS: Both ILs incorporation induces changes in the surface roughness, surface wettability and on the electrical properties of the films. An increase in the β -phase-content and crystallinity was observed upon the incorporation of 5wt.% IL, indicating that ILs, at this content, act as a nucleating agent for polymer crystallization process. Only the [Ch][DHP]/P(VDF-TrFE) samples proved to be biocompatible and able to support H9c2 cell adhesion and proliferation. The inclusion of GO influences the microstructure, surface roughness and the P(VDF-TrFE) crystallization process. The surface wettability of the composites remains almost the same after GO incorporation. The Young modulus and the electrical properties were both increased by the GO incorporation, and all GO/P(VDF-TrFE) samples proved to be biocompatible. Apart from it, it was observed that the H9c2 cell adhesion and proliferation was influenced by the surface charge of PVDF films, with the positively charged surfaces promoting higher myoblast adhesion and proliferation either at static or dynamic conditions.

DISCUSSION & CONCLUSIONS: The noncytotoxicity of the [Ch][DHP]/P(VDF-TrFE) and GO/P(VDF-TrFE) composites, and H9c2 cell proliferation prove the suitability of these materials for cardiac regeneration. This work also demonstrates that mechano-electric stimuli are essential to improve tissue regeneration. Thereby, electroconductive biomaterials represent a promising approach for cardiac regeneration strategies.

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R.M.Meira,et al.,ACS Appl.Polym.Mater, 2019

Keywords: In vitro microenvironments, Electroactive materials

OP-138

Targeting mechanosignaling to modulate the immune environment for bone repair

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INTRODUCTION: It is well-established that bone repair is highly sensitive to loading magnitude, where low/moderate strains accelerate healing, while high strains result in delayed/non-healing associated with heightened inflammation. However, little is known about the role that mechanical cues play in driving the local immune response in the early fracture haematoma. Interestingly, two second messengers, known to be mechanoresponsive in bone cells, have been shown to be involved in macrophage polarisation: calcium (Ca^{2+}) and cyclic adenosine monophosphate (cAMP). In this study, we investigated the potency of two therapeutics, Ca^{2+} channel antagonist GSK2798745 (GSK) and cAMP agonist Forskolin, to modulate macrophage phenotype via blocking mechanotransduction in a high load inflammatory environment or alternatively mimicking a low load environment, establishing a regenerative setting for bone healing.

METHODS: Human THP-1 monocytic cells were differentiated into macrophages (hM ϕ) with 100nM PMA at 10^6 cells/ml in complete RPMI medium for 3 days. To evaluate the potency of both drugs, hM ϕ were first pre-treated for 2 hours with 10nM, 100nM, and 1uM of GSK to inhibit TRPV4 activity or 0.1uM, 1uM, and 10uM of Forskolin to activate cAMP signalling. Pre-treatment of GSK and Forskolin were followed by a 24-hour differentiation into pro-inflammatory M1 phenotype with LPS (100ng/mL) and IFN- γ (20ng/mL) or an additional 24-hour treatment in naïve M0 macrophages, respectively. Real-time PCR and ELISA were performed at the end of both experiments to quantify mRNA and cytokine levels.

RESULTS: Analysis of gene expression confirmed downregulation of M1 markers such as CD80 and IL-1 β , following GSK treatment. Similarly, a partial inhibition of pro-inflammatory markers IL-6 and TNF-alpha was observed both at a gene and cytokine expression level. Additionally, GSK treatment promoted gene and cytokine expression of angiogenic marker VEGF-A. Analysis of gene expression suggested a transition into a hybrid M1/M2 phenotype following Forskolin treatment, with downregulation of MRC1 and upregulation of CCL22. Increasing dose of Forskolin was associated with increased gene expression and secretion of anti-inflammatory IL-10 and angiogenic VEGF-A markers.

DISCUSSION & CONCLUSIONS: Results suggest that modulation of the early immune environment can be achieved with newly identified 'mechanotherapeutics'. Upon examining the performance of both drugs, GSK was shown to partially impede M1 polarisation following LPS treatment, whilst Forskolin promoted a more pro-regenerative phenotype in naïve M0 macrophages. Interestingly, both drugs enhanced the secretion of VEGF-A, key angiogenic marker known to direct vascularisation in the fracture haematoma. Future work will aim to support this analysis performing functional assays including angiogenesis and osteogenesis.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Immunity / immunomodulation / macrophage

OP-139

Immunomodulation in tissue engineering application: the role of extracellular vesicles from diabetic adipose derived-stem cells

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INTRODUCTION: Recent studies have highlighted the immunomodulatory properties of adipose tissue derived stem cell extracellular vesicles (ADSC EVs) in tissue repair. Obesity is usually associated with chronic and aberrant inflammation and oftentimes is a co-morbidity of type 2 diabetes. While ADSC EVs are well studied in tissue engineering application, the immunomodulatory properties of ADSC EVs derived from diabetic patients is uncharacterized. The objective of this study is to evaluate the composition and immunomodulatory properties of ADSC EVs derived from diabetic individuals. **METHODS:** Age and sex matched human ADSCs and type 2 diabetic ADSCs (d-ADSCs) were purchased from Lonza. EVs were isolated from conditioned medium from ADSCs and d-ADSCs (ADSC EVs, d-ADSC EVs respectively). For EV characterization, immunoblotting was performed to assess exosomal markers (CD63 and CD81) expression. Nano particle analysis was performed to measure EV particle size distribution and microRNA sequencing was performed to compare the miRNA composition in ADSC and d-ADSC EVs. To evaluate the immunomodulatory effects of EVs, primary bone marrow derived macrophages were isolated from 8-week-old mice. The effects of ADSC EVs and d-ADSC EVs on primary macrophages under M1 or M2 polarization was evaluated by qRT-PCR and ELISA in vitro. In addition, the in vivo effects of both EVs were characterized in a rat calvarial defect model at days 1, 3 and 7 post wounding using qRT-PCR, ELISA and immunohistochemistry (IHC). Further, rat calvaria were harvested at 4- and 8- weeks to evaluate bone formation using μ CT and IHC. For statistical analysis, ANOVA and Tukey's ad- hoc test was used for statistical calculations with a confidence interval of 95%.

RESULTS: Our results indicate that the physical characteristics of the EVs remain unchanged between ADSC and d-ADSC EVs. ADSC EVs and d-ADSC EVs altered the expression levels of M1 markers and increased M2 marker Arginase 1 expression in M1 macrophages at similar levels in vitro. In rat calvaria model, differed inflammatory response was observed in EV treated calvaria compared to control (PBS) group. The iNOS/Arg1 positive cells ratio was reduced in ADSC and d-ADSC EV groups, and both EV groups significantly promoted bone formation at 8 weeks.

DISCUSSION & CONCLUSIONS: ADSC EVs and d-ADSC EVs possess similar levels of anti-inflammatory properties in vitro and in vivo indicating that MSCs from adipose tissues of diabetic patients can potentially serve as a source for EV therapeutics for immunomodulatory applications in tissue repair.

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Keywords: Extracellular vesicles, Immunity / immunomodulation / macrophage

OP-140

Immunomodulatory effect of Matrix Bound Nanovesicles (MBV) upon myeloid precursor cells

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INTRODUCTION: Matrix bound nanovesicles (MBV) are extracellular vesicles inherent to the extracellular matrix (ECM) known to have an immunomodulatory effect on myeloid immune cells, more specifically macrophages. This modulation has been characterized by a decrease of the inflammatory phenotype in vitro and to a mitigation of disease symptoms in in vivo models of psoriasis, rheumatoid arthritis, and optic nerve injury. However, the effect of this immunomodulation upon myeloid progenitor cell and their ability to regulate adaptative immune responses has not been investigated.

METHODS: In the present study, mice were immunized with the pneumococcal vaccine and injected intraperitoneally with either 1e11 MBV or 1 µg of methotrexate weekly for 5 weeks, following either a mild infection intranasally or a septic infection intraperitoneally. After intranasal infection, bone-marrow macrophages were isolated and challenged with pneumococcal polysaccharide (PPS) or E.Coli lipopolysaccharide (LPS) ex vivo and the triggered inflammatory response was compared.

RESULTS: MBV did not affect the ability of mice to produce specific IgG and IgM against PPS both before and after intranasal infection with 1e07 CFU of Streptococcus Pneumoniae, contrary to the methotrexate group. Of note, 10 days after intranasal infection, vaccine + MBV group showed a lower infiltration of CD4⁺ cells and a lower fibrotic collagen deposition in lungs compared to vaccinated mice. Additionally, lethal intraperitoneal infection with 1e11 CFU caused total death in all groups after 2 days except for those treated with vaccine + MBV, which showed a 50% survival. Macrophages from groups immunized with the vaccine only and vaccine + MBV showed a stronger response to PPS in terms of IFN γ production, whereas only those macrophages isolated from vaccine + MBV group showed a higher IFN γ and IL-23 expression when exposed to LPS. These results indicate a change in the acquired phenotype or “innate memory” elicited by MBV on progenitor myeloid cells. This modulation on myeloid progenitor cells was confirmed by injecting MBV in healthy mice and isolating macrophages after 48 hours, which showed a lower response to LPS challenge in terms of IFN γ production when compared to sham mice-derived macrophages. Similarly, bone marrow cells exposed to MBV ex vivo during differentiation resulted in macrophages with different phenotype than macrophages exposed to MBV after terminal differentiation.

DISCUSSION & CONCLUSIONS: These results suggest that MBV modulate the acquired memory of progenitor myeloid cells, eventually modulating the immune response. Future studies at transcriptomic and epigenetic level are required to more fully understand the mechanisms of these effects.

Keywords: Immunity / immunomodulation / macrophage, Extracellular vesicles

OP-141

Cellular Interactions at the Decellularised Scaffold:Tissue Interface in an Ex-Vivo Organ Culture Model

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INTRODUCTION: Modulation of the cellular response to decellularised biological scaffolds towards an immuno-regulatory (M2) phenotype has been suggested to be a determinant of regenerative outcomes in vivo¹. A key role in recellularisation of functional decellularised heart valve scaffolds was revealed for macrophages during 6-month² and 12-month³ in vivo ovine models. CD163+ (M2) macrophages were shown to pioneer the initial cellular response to porcine decellularised bladder scaffold in a human tissue organ culture model⁴. The aim of this study was to develop an ex-vivo organ culture model for decellularised cardiovascular scaffolds, that could replicate the initial innate and stromal cell response seen at the in-vivo tissue:decellularised scaffold interface.

METHODS: An interface was established between decellularised porcine pulmonary artery scaffolds and native ovine pulmonary artery. The constructs were maintained in culture for 0, 2, 6, and 11 days (n = 4 at each time point). Haematoxylin and eosin (H&E) staining, and immuno-histochemistry, was used to characterise the cellular response at the tissue:scaffold interface; using primary antibodies for macrophages, smooth muscle cells, proliferating cells, progenitor cells, endothelial cells and connective tissue growth factor. Manipulation of the ovine ex vivo cellular response was investigated by exposing decellularised scaffolds to two concentrations of a carbodiimide cross-linker prior to organ culture.

RESULTS: Observation of H&E stained histological sections revealed a time dependent infiltration of cells into the non-crosslinked scaffolds. This was a heterogeneous population, expressing markers for stromal and progenitor cells. A striking number of cells within the scaffold and at the tissue:scaffold interface were CD163+ (M2 macrophage marker). Analysis of the cellular response towards the cross-linker treated decellularised scaffolds revealed that the ovine cellular response was attenuated in comparison to the non-crosslinked scaffolds. The cells appeared to collect at the interface, similar to the encapsulation response, rather than crossing into the scaffold.

DISCUSSION & CONCLUSIONS: The striking CD163+ cell presence, in combination with the recruitment of site specific stromal cells and progenitor cell types, indicates that this ex-vivo model may provide a valuable tool for investigating the mechanisms of an early regenerative cellular response. Further investigation of the cell infiltrate and mechanisms of recruitment and polarisation of macrophages at the scaffold interface is required.

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Keywords: Decellularised matrices, Cardiovascular

OP-142

Extracellular Matrix Production by Primary Human Adipose-Derived Vascular Smooth Muscle-like Cells Interacting with Monocytes on a Nanofibrous Polyurethane Scaffold

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INTRODUCTION: Commercial vascular grafts with small diameters (<6 mm) continue to have poor long-term patency rates and mismatched mechanical compliance. Grafts engineered from patient-derived cells with the potential to remodel and grow within the recipient may provide a tenable solution for reconstructive cardiovascular surgery in pediatric populations.

METHODS: Adipose stromal cells (ASCs) were isolated from human tissue with enzymatic digestion, centrifugation and magnetic cell-sorting. P5 ASCs were differentiated into VSMC-like cells (ASC-VSMCs) with TGF- β 1 and retinoic acid. Human monocytes were isolated from whole blood through a Histopaque[®] density gradient and centrifugation. Cells were co-seeded through a two-stage process at a ratio of 4:1 (ASC-VSMC: monocyte) on nanofibrous polyurethane scaffolds with a surface area of 8.8 cm² at a density of 70,000 cells/cm². Cultures were supplemented with 100 μ M of sodium ascorbate, previously observed to enhance elastin and glycosaminoglycan (GAG) production following four weeks of culture in microporous constructs[1]. Samples were harvested weekly over the duration of one month. Immunostaining was carried out for α -smooth muscle actin, smoothelin, collagen I, III, and IV, and elastin. Hydroxyproline analysis was used to quantify collagens; sulphated GAGs were measured with a dimethylmethylene blue assay; and a Fastin assay was used for assessing elastin production.

RESULTS: One week post culture, the ASC-VSMCs were observed to form a confluent layer in alignment with the nanofibrous scaffold. Elastin production doubled and collagen production increased between 7 and 11-fold from the first to the fourth week of co-culture. GAG and DNA quantity initially increased and then plateaued after week 3.

DISCUSSION & CONCLUSIONS: In this co-culture system, collagen and elastin - two major ECM proteins - were upregulated in a reproducible manner with cells obtained from different human host tissues, of variable baseline health. The results have also been validated both with direct quantification through biochemical assays and semi-quantitative immunofluorescent imaging. ECM production trends begin to plateau at week 3, indicating a switch from tissue growth to a remodeling phase. Further work is on-going to investigate the remodeling and incorporate mechanical simulation within a pulsatile bioreactor for the accelerated production of tissue in tubular form.

Given the favorable results in the current work, it is anticipated that the biochemical factors from monocytes coupled with mechanical stimuli will generate engineered tissue with a functional and comprehensive ECM for the formation of arterial grafts.

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Keywords: Microenvironment and niche engineering, Vascular systems / vascularisation and heart

OP-143

Encapsulation and Sustained Release of Functionally Engineered Extracellular Vesicles in Peptide Based Hydrogel

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INTRODUCTION: Mesenchymal stem cell (MSC) derived extracellular vesicles (EVs) in their naïve and engineered forms have a defined therapeutic potential. These functional EV therapeutics requires spatial and temporal control of EV delivery to be translationally relevant for tissue engineering applications. The objective of this study was to evaluate the ability of a self-assembling leucine zipper (LZ)-based peptide hydrogel with EV tethering motifs to encapsulate and release functionally engineered EVs (FEEs) and test it in a model of bone repair.

METHODS: The binding characteristics of osteoinductive FEEs to control, RGD, DGEA, GFPGER peptide containing LZ hydrogel was evaluated using quantitative fluorescence-based assay. The engineered EVs were encapsulated in LZ hydrogels containing the RGD tethering peptide and release profiles in 7% w/v LZ hydrogels was evaluated over a period of 21 days. The structural and functional integrity of the encapsulated EVs was evaluated by nano particle tracking analysis (NTA), qRTPCR for osteoinductive genes as well as by Alkaline phosphatase activity and calcium deposition assays. The in vivo functionality of the engineered EVs encapsulated in LZ hydrogels was evaluated in a rat calvarial defect model followed by μ CT and immunohistochemistry. ANOVA followed by Tukey's post hoc analysis was used for statistical significance ($P < 0.05$)

RESULTS: In in vitro experiments, the FEEs bound dose dependently and saturably to the tethering peptides. LZ hydrogels containing RGD peptide were able to encapsulate, tether and retain the FEEs over a period of 21 days while maintaining the structural integrity and osteoinductive functionality of the EVs. When employed in a calvarial defect model in vivo, LZ-RGD hydrogels containing the FEEs enhanced bone regeneration significantly compared to controls.

DISCUSSION & CONCLUSIONS: These results show that Engineered EVs can be tethered to peptide-based biomaterials such as LZ hydrogels to promote tissue repair. Results also provide a prelude to the possible use of this technology for controlled delivery of EVs for other regenerative medicine applications when the peptide/hydrogel properties are altered suitably.

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Keywords: Biomaterials, Hydrogels and injectable systems

OP-144

Nitric Oxide (NO) Loaded Gelatin Nanoparticles as Antimicrobial Therapeutics for the Treatment of Respiratory Infections

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INTRODUCTION: Acute respiratory infections (ARIs) are responsible for 4.25 million deaths globally each year¹. Given the rise in antimicrobial resistant pathogens², the treatment of ARIs necessitates alternatives to antibiotics. Nitric oxide (NO) is an effective broad-spectrum antimicrobial, which is endogenously produced by the immune system in response to pathogens. NO has a multimechanistic mechanism of action and therefore does not lead to microorganisms developing resistance. Delivery of NO is challenging, however, as it is a highly reactive gas with a short half-life. In this study, we report on the fabrication of gelatin nanoparticles (GNPs) as drug delivery vehicles for the controlled release of NO. The efficacy of these NO loaded GNP (GNP/NO) were evaluated for their antimicrobial efficacy against 4 medically relevant pathogens.

METHODS: GNPs were fabricated using the two-step desolvation method, and loaded with N-diazoniumdiolates (GNP/NO). Their physicochemical properties were assessed using dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Chemiluminescence was used to determine the NO payload and kinetics of release. The antimicrobial efficacy was tested over a 24 h period against "Escherichia coli", "Staphylococcus aureus", "Pseudomonas aeruginosa" and "Candida albicans". The cytotoxicity of GNP/NO were evaluated against L929 cells using a standard ISO-10993 protocol.

RESULTS: GNPs were synthesised at pH 3.5 and found to produce homogenous spherical particles ~225nm in diameter by DLS and SEM analysis. FTIR spectrum displayed peaks characteristic of N-diazoniumdiolates at 1380 and 1278 cm⁻¹ confirming its successful loading into GNPs.

Chemiluminescence analysis showed GNP/NO to release an initial NO burst, followed by a sustained release over 24 h. Antimicrobial testing revealed GNP/NO to cause a significant reduction (up to 3 log) in remaining viable organisms after 4 h, and 24 h, against all tested pathogens. Cytotoxicity assays demonstrated GNP/NO to maintain over 70% cell viability.

DISCUSSION & CONCLUSIONS: We have demonstrated the successful development of NO loaded GNPs with excellent antimicrobial capabilities within a cytocompatible range. Further investigation is warranted to assess the "in vivo" cytocompatibility of the GNPs, ensuring their feasibility for use as an effective treatment for ARIs.

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Keywords: Drug delivery, Infection

OP-145

Wireless electrostimulation for cancer treatment: an integrated nanoparticle/coaxial fiber mesh platform

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INTRODUCTION: Controlled drug delivery systems are key to develop new cancer treatment strategies, as cancer is the leading cause of death in many developed countries. The use of controlled drug delivery systems allows to provide a localized therapy and avoiding systemic toxic effects, as lower amounts of drug administration would be required.

METHODS: A wireless electrostimulation system was developed for the treatment of breast and prostate cancer composed of two components: poly(3,4-ethylenedioxythiophene) (PEDOT) nanoparticles (NPs) loaded with anticancer drug curcumin (CUR), encapsulated in biodegradable coaxial poly(glycerol sebacate)/poly(caprolactone) (PGS/PCL) fibers. Both NPs and fibers were fully characterized. The PEDOT NPs allowed the controlled delivery of CUR using specific applied potentials. The coaxial fibers were employed to control the systemic release of the PEDOT NPs, depending on lipolytic activity.

RESULTS: A controlled release of CUR from NPs was achieved by electrostimulation, achieving 79% of CUR released at an electrical potential of -1.5 V. Biodegradation of the fibers was assessed using lipase, resulting in a sustained release of NPs. Wireless electrostimulation of PC-3 cancer cells using CUR/PEDOT NPs was successfully performed, resulting in 67% decrease in cancer cell viability, caused by the controlled release of CUR from the PEDOT NPs.

DISCUSSION & CONCLUSIONS: We have successfully produced PGS/PCL coaxial fibers, with embedded electroresponsive PEDOT NPs, which were loaded with the anticancer drug CUR. PC-3 and MCF7 cancer cell lines internalized CUR/PEDOT NPs in 24h, with no perceived cell death or alterations in cell morphology in the absence of electrical stimulation. Most importantly, wireless electrostimulation of cancer cells using CUR/PEDOT NPs was successfully performed, resulting in a very significant decrease in cancer cell viability, caused by the controlled release of CUR from CUR/PEDOT NPs. Overall, our results show the potential of using wireless electrostimulation of drug-loaded NPs for cancer treatment, using safe voltages for the human body, and ensuring the delivery of the anticancer drug in a highly controlled way. Additionally, our results highlight the potential of using FDA approved materials to create a transdermal implant that would act as a reservoir of the

NPs to be delivered in a sustained manner to the patient.

ACKNOWLEDGEMENTS: This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project Belive (PTDC/EMD-EMD/30828/2017), iBB (UIDB/04565/2020 and UIDP/04565/2020), i4HB (LA/P/0140/2020) and PhD scholarship (SFRH/BD/145057/2019). The authors would like to thank Professor Ana Marques, Professor Rogério Colaço and Professor Luís Fonseca for their contributions.

Keywords: Cancer, Drug delivery

OP-146

Click Chemistry Improves the Stability of dECM Hydrogels and Provides an Angiogenic Environment for Vocal Fold Regeneration

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INTRODUCTION: Decellularized extracellular matrix (dECM) hydrogels show promise for vocal fold (VF) tissue regeneration due to the presence of molecules that stimulate wound healing [1]. However, current VF dECM hydrogels suffer from rapid degradation and their potential for functional angiogenesis is unknown [2]. In this work, we show click tetrazine ligation of VF dECM with the slower degrading natural polymer alginate can improve stability of dECM hydrogels while retaining biocompatibility and stimulating the formation of capillary networks.

METHODS: VF dECM was extracted from porcine larynges using a detergent-free protocol, solubilized with pepsin, and functionalized with norbornene by carbodiimide crosslinking. Alginate was functionalized with methyltetrazine. dECM-only hydrogels were used as controls. Stability and degradation assays were performed by incubating hydrogels in hydrolytic (PBS) and enzymatic (collagenase I) conditions for 30 days.

For cellular studies, immortalized Human Vocal Fold Fibroblasts (HVFF) or primary Human Umbilical Vein Endothelial Cells (HUVECs) were encapsulated in 3D click and dECM-only hydrogels. HUVEC studies included two culture conditions: with (+) or without (-) vascular endothelial growth factor (VEGF) in the media to determine if exogenous factors are needed to support capillary formation. Live/Dead viability assays were performed at Day 14.

RESULTS: Click (1.6% dECM, 0.4% Alginate) and pepsin-solubilized dECM-only hydrogel controls both formed within 30 min at 37 °C.

Under both hydrolytic and enzymatic conditions, click hydrogels lost mass more slowly than dECM-only controls. Click hydrogels retained mass up to 72% (hydrolytic) and 22% (enzymatic) at day 30. dECM-only controls retained up to 8% of the mass under hydrolytic conditions, and degraded completely under enzymatic conditions at day 15.

For cellular studies, HVFF and HUVECS showed over 90% viability in both hydrogels at all times. Click hydrogels did not contract, but dECM-only hydrogels exhibited up to 90% contraction by day 14. In both hydrogels, HUVECs formed network-like structures in whether or not media contained VEGF.

DISCUSSION & CONCLUSIONS: Our findings indicate that click crosslinking with alginate augments the stability of VF dECM hydrogels without impacting cell biocompatibility. The formation of HUVEC network-like structures in media without VEGF indicates dECM retained molecules that aid in capillary formation for functional repair. Future work will include angiogenetic markers such as tight junctions and basement membrane to confirm capillary maturity.

ACKNOWLEDGEMENTS: Financial support was received from the Natural Sciences and Engineering Research Council of Canada RGPIN-2018-03843 and NSERC ALLRP 548623-19.

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[2] Wan-Chiew N et al. Polymers 2021;13(16), 2619.

Keywords: Decellularised matrices, Composite materials

OP-147

Microfluidic Encapsulation of Human Bone Mesenchymal Stromal Cells (hBMSCs) in Microgels for Cartilage Regeneration

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INTRODUCTION: Microgels are defined as microscale particles of conventional bulk hydrogels. With enhanced surface area to volume ratio, microgels provide biocompatible scaffolds for encapsulated cells with increased nutrient/gas exchange and greater cell-cell interactions to promote the formation of engineered tissues. In cartilage regeneration, microgels laden with human bone marrow-derived mesenchymal stromal cells (hBMSCs) have achieved promising outcomes in mimic cartilage tissues production. Recent findings revealed that encapsulated cells tend to migrate toward the surface of the microgels after a few days and eventually deposit their cartilaginous matrix in the voids formed between the microgels. However, the mechanism behind this phenomenon has not yet been clear. Microgel size has been hypothesized to be one of the key factors influencing this phenomenon. Our work has focused on elucidating the impact of microgel size on cell migration and cartilage tissue formation.

METHODS: Devices: We previously developed a microfluidic system assembled from laboratory consumables to produce gelatin-based microgels. This study modified the device nozzle part size and optimised flow rate combinations of the oil fluid versus the hydrogel fluid to produce microgels of three different sizes.

Cells: hBMSCs were encapsulated within microgels and subsequently induced towards chondrogenic differentiation for engineered cartilage production.

Analysis: Cell viability and migration within varying-sized microgels were evaluated and histological staining was assessed to visualise the newly formed cartilage quality.

RESULTS: Gelatin-PEG based microgels with high uniformity and controllable size (200 – 600 µm) were fabricated. Three microgel sizes 500 µm, 350 µm, and 180 µm were used to investigate cell viability and migration. Live/dead staining showed high viability in all conditions. At 3 days, more cells reached the microgel surface in the 180 and 350 µm microgels, compared to 500 µm microgels but on day 7 most cells were on the microgel periphery, regardless of size. Cartilage tissue structure was also affected by microgel size, a uniform cartilage structure was present in voids between 350 µm microgels, whereas cells loaded with 500 µm microgels showed sparse cartilage accumulation. The 180 µm microgel structure collapsed, leaving a contracted cartilage aggregate.

DISCUSSION & CONCLUSIONS: This work fabricated a microfluidic system that enabled microgel production at a wide range of sizes (200 – 600 µm). Microgels of varying sizes were used to better understand the connection between cell migration and cartilage formation in microgel constructs, showing an optimum microgel size of 350 µm. These insights can be applied to improve the quality of engineered cartilage for cartilage regeneration.

Keywords: Hydrogels and injectable systems, Stem cells - general

OP-148

Osteoniche-on-a-3D printed chip for determining metastatic invasion of patient-derived cancer cells

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INTRODUCTION: Malignancy accounts for 90% of cancer associated mortality. Bone tissue is a frequent site for many metastatic cancers. Bone microenvironment has critical structural and functional factors influencing tissue homeostasis to metastasis. Extracellular matrix mimetic test bed to predict invasion potential and homing choices of cancer cells could help in deciphering the osteotropism.

METHODS: We describe the development of a 3D printed microfluidic platform for the quantification of invasion and extravasation of cancer cells towards the bone niche. This is achieved via incorporation of a tissue-specific hydrogel (osteomatrix) generated by decellularization protocol in the chip. The microfluidic chip was fabricated by stereolithography (SLA). The bidirectional interactions between cancer cells and the bone niche/micro-environment are limited by immortalized cell lines owing to their altered genetic content and receptor expression. To closely mimic the in vivo conditions, the primary cancer cells from patients were isolated and characterized for invasion studies. In addition, we analyzed the role of macrophage populations in tumor invasion through co-culture of cancer cells and activated macrophages (in presence of LPS or IL-4).

RESULTS: The physico-biochemical characterization and histological studies showed the efficiency of the tissue decellularization protocol. Further, osteogenic differentiation of adipose-derived mesenchymal stem cells on osteomatrix without external factors proved by osteogenic gene expression analysis and alkaline phosphatase (ALP) enzyme activity suggested tissue ECM functionality. Next, we compared the invasion potential of patient-derived oral cancer and breast cancer cells in chip. The quantitative and comparative assessment of osteo-specific tumor invasion on chip was correlated with the clinical data. In comparison to standard collagen gel, a proinvasive effect of macrophages was observed in the presence of osteomatrix. Finally, the paclitaxel drug treatment demonstrated an effect on cancer cell migration ability, viability, and morphology in chip.

DISCUSSION & CONCLUSIONS: The differential invasion potential and drug sensitivity between the patients confirms the cancer-specific functionality of our model system. Our 3D printed microfluidic device with osteomatrix serves as an effective platform for screening anti-metastatic drugs within physiological-like conditions validating its potential use in personalized medicine.

Keywords: Decellularised matrices, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

OP-149

Electrically Conductive Biomaterials for Controlling Stem Cell Fate

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INTRODUCTION: Electrical stimulation, a physical stimulation which can be delivered via a conductive biomaterial interface, directs human mesenchymal stem cell (hMSC) differentiation towards different cell tissue types.[1] Electrical stimulation conditioning offers a promising approach in directing stem cell fate. Conductive biomaterials are commonly used to provide either a passively conductive substrate, or actively provide 'smart' electrical stimulation of stem cells for tissue engineering. However, the mechanisms in which cells transduce these electrical signals into specific phenotype differentiation are poorly understood, restricting the intelligent design of stimulation protocols for targeted differentiation.

METHODS: How the stem cells transduce an electrical signal into a biological response is explored using two types of charge injection (ionic or photocurrent) via conductive biomaterials. Immediate changes in the stem cells during and post-stimulation is characterised, using live cell bio-AFM for morphological and biomechanical changes, complemented with standard biological characterisation. The advanced bioAFM technique delivered unprecedented intracellular biomechanical information of live cells undergoing simultaneous electrical stimulation.

RESULTS: Live bioAFM revealed transient changes in hMSC area, volume, and stiffness during and post-stimulation. Stimulation also induced changes in focal adhesions and YAP translocation behaviour in the stem cells over short (<24h) periods of time. Using advanced bioAFM mechanical analysis changes in actin bundles, perinuclear region, and nuclear cap could be quantified as a response to stimulation.

DISCUSSION & CONCLUSIONS: For the first time we have characterised the transient mechanical response of hMSC to electrical stimulation via a conductive polymer substrate, and related that to controlling stem cell differentiation towards osteogenesis. The knowledge gained from this study helps to further the intelligent design of stimulation parameters for targeted differentiation outcomes when using a conductive biomaterial.

REFERENCES: [1] Gelmi, A., Schutt, C. E., Stimuli-Responsive Biomaterials: Scaffolds for Stem Cell Control. Adv. Healthcare Mater. 2020, 10, 2001125.

Keywords: Stem cells - general, Biomaterials

OP-150

Use of pectin for the spray delivery of mesenchymal stromal cells for treating brain injury

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INTRODUCTION: Current therapies for brain injury aim to limit further damage occurring via secondary injury pathways as a response to the initial trauma. Animal studies have shown improved neurological outcomes after injury following mesenchymal stromal cell (MSC) transplantation¹, however successful delivery of these cells is limited by the delivery route. Cells delivered intravenously show poor migration to the site of injury, resulting in a large loss of therapeutic cells.

We hypothesise that delivering these cells locally by spraying within a hydrogel-forming pectin solution will enhance therapeutic outcomes by delivering and retaining a higher proportion of the cells at the injury site. Pectin is cross-linkable with calcium, enabling cells to be sprayed in solution.

METHODS: Four grades of citrus pectin were selected with different degrees of methylation (DM) and amidation (DA): CU-701 and CU-L from Herbstreith & Fox have 37% and 28% DM respectively and LM-102 and LM-104 from CP Kelco have 19% and 20% DM, and 30% and 27% DA respectively. The syringe based Mucosal Atomisation Device (MAD), was chosen due to the simplicity of loading cells into a syringe for spraying. Cytotoxicity testing on 1% pectin gels formed with 100 mM Ca²⁺ was carried out following the ISO 10993-5 using SH-SY5Y human neuroblastoma cells. Human MSC density for spraying was optimised, before assessing the effect of spraying in pectin on the phenotype of MSCs by flow cytometry. Preliminary studies to understand the therapeutic potential of the sprayed therapeutic cells with pectin have been carried out using in vitro damage models of excitotoxicity and oxidative stress using SH-SY5Y neuroblastoma cells.

RESULTS: Pectin demonstrated a high biocompatibility in the cytotoxicity testing, with >80% metabolic activity observed in all conditions after 24 hours. High viability was also maintained following spraying MSCs (>80%). CU-701 and LM-102 were selected as the most promising grades of pectin following the results of the flow cytometry analysis, which showed that MSCs retained the expression of CD90, CD73, and CD105 following spraying within pectin, and remained negative for CD34, CD11b, CD19, CD45 and HLA-DR. These two pectin grades also maintained the highest cell viability in the excitotoxicity and oxidative stress damage models.

DISCUSSION & CONCLUSIONS: Our results indicate the simple syringe-based MAD sprayer is appropriate for the delivery of MSCs within pectin. CU-701 and LM-102 are the most promising grades of pectin for cell delivery, based on viability and flow cytometry results.

REFERENCES: (1) Volkman et al., Stem cells, volume 35, 1867-1880, 2017

Keywords: Multipotent (mesenchymal) stem cells, Biomaterials

OP-151

The effects of ST2 mesenchymal stem/stromal cell secretomes on the growth and differentiation of E14TG2a embryonic stem cells during early development

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INTRODUCTION: Mesenchymal stem/stromal cell (MSC) secreted factors have multiple paracrine effects on cells, including promoting cell survival, proliferation and differentiation, neurogenesis, angiogenesis, immunomodulation and tissue formation. However, the roles of MSCs in regulating embryonic stem cells (ESC) are currently unknown. Here, we use classical 2D adherent ESC cultures and 3D ESC-derived mouse gastruloid cultures to investigate the regulatory influence of MSC culture conditioned media in serum-supplemented and serum-free conditions harvested over time (MSC CM) on ESCs.

METHODS: ST2 cells were seeded into T75 flasks and left to adhere overnight, before replacing standard medium with serum-supplemented or serum-free medium, harvested after 24-72h. 2D cultures of E14TG2a ESCs were incubated with MSC CM or seeded into culture plates that had been pre-coated with MSC CM. 3D E14TG2a cultures were established (van den Brink et al., 2014) and then treated with MSC CM. Cell viability, proliferation, and differentiation were assessed using MTS assays, LIVE/DEAD staining, and immunolabelling. All data presented is derived from triplicate independent experiments.

RESULTS: In 2D experiments, treatment with 24h MSC CM increased ESC proliferation, while 72h MSC CM inhibited ESC proliferation. Coating culture plates with MSC CM increased ESC proliferation. In 3D experiments, treatment of ESC with serum-free MSC CM resulted in the formation of gastruloids in the absence of Chiron, a Wnt activator. At 120h, these MSC CM-induced gastruloids exhibited symmetry breaking and elongation. At 168h, over 80% of the gastruloids exhibited cardiomyocyte features, i.e., beating cells. The addition of Chiron to serum free MSC CM accelerated gastruloid maturation at 96h, but inhibited cardiomyocyte induction at 168h. Treatment with serum supplemented MSC CM without Chiron did not induce gastruloid formation or cardiomyocyte differentiation. Gastruloid formation was seen when Chiron was added to serum-supplemented MSC CM cultures; however, there was reduced elongation compared to Chiron-treated serum-free MSC CM-induced gastruloids.

There was an increase in immunopositivity for the Troponin 1, GATA-6, Brachyury and Sox-2 differentiation in MSC CM-induced gastruloids.

DISCUSSION & CONCLUSIONS: These novel findings suggest that MSC secreted factors regulate ESCs. Under the right conditions, this regulatory activity can be used to determine ESC fate and promote ESC differentiation into beating cardiomyocytes. Hence, this study opens the possibility of using the paracrine activity of MSCs both to better understand the regulation of gastrulation and also to enhance ESC-derived therapies for cardiovascular pathologies.

Keywords: Developmental biology, Differentiation

OP-152

A 3D in-vitro model of macrophages in the subcutaneous tissue for bioavailability assessment

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INTRODUCTION: Many novel biopharmaceuticals are injected subcutaneously (SC) due to the advantages offered by this method. The drug development process of SC biopharmaceuticals relies heavily on the use of animal models to determine their bioavailability and interactions in the SC tissue. This has proven to be ineffective due to interspecies SC physiological and anatomical differences. To address this gap, we propose to develop a 3D in-vitro model of the SC tissue to test drug bioavailability with a particular focus on the role of macrophages in drug absorption.

Macrophages are thought to be a site of first pass catabolism, and their role in drug absorption is also likely to be impacted by their phenotype, which changes depending on the inflammatory state of SC tissue. For instance, in obesity mediated metabolic dysfunction, SC adipose tissue is known to adopt an inflammatory state leading to the recruitment of more M1 pro-inflammatory macrophages. How this might impact SC bioavailability has not yet been investigated.

METHODS: A biomimetic hydrogel composed of collagen and hyaluronic acid was developed for the 3D co-culture of macrophages and adipocytes. Human mesenchymal stem cells were cultured on the 3D hydrogel and differentiated to adipocytes. Macrophages were differentiated in-vitro from primary monocytes isolated from human blood before seeding into the 3D hydrogels. For co-culture, macrophages were seeded with the adipocytes and the co-culture conditions optimised.

RESULTS: Macrophages seeded on the 3D hydrogels were shown to survive and polarise in response to stimuli in the media such as IL-4 or lipopolysaccharide (LPS). This was assessed using immunostaining and analysis of cytokine release with ELISA. It was revealed that the soft mechanical properties of the hydrogel promoted an M0 polarisation for the macrophages except in the presence of LPS. When co-cultured, the interaction of macrophages and adipocytes in the 3D hydrogel was characterised using immunostaining, live imaging, and the analysis of released cytokines. For further analysis a protocol to isolate the cells for FACS is being developed and the interactions of the co-culture with drug molecules/antibodies will be assessed.

DISCUSSION & CONCLUSIONS: A 3D biomimetic model of the subcutaneous tissue with macrophages and adipocytes will enable us to investigate in detail the role of macrophages in drug absorption from the SC tissue and how this is affected by their interaction with adipocytes and changes in inflammatory state of the adipose tissue.

ACKNOWLEDGEMENTS: Animal free research UK, the Daphne Jackson Trust, Elizabeth Blackwell Institute, the University of Bristol

Keywords: In vitro microenvironments, Biologics and growth factors

OP-153

Understanding The Mechanisms Of Action Of Collagen-Based Wound Dressings To Promote Healing

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INTRODUCTION: When a wound occurs and fails to proceed through the physiological phases within 3 months it is referred to as a chronic wound [1,2]. The rising prevalence of chronic wounds puts increasing pressure on health services worldwide, calling for the understanding of the mechanisms of action of available dressings to promote healing [3,4].

Collagen-based dressings offer numerous beneficial properties to chronic wounds treatment as collagen is the main component of skin ECM [5,6]. Commercially available collagen-based wound dressings represent a large class of dressings. However, there is a lack of comparative data to differentiate between these dressings and understand their mode of action.

METHODS: The dressings chosen for analysis were oxidized regenerated cellulose (ORC)/Collagen and Collagen/ORC/silver, P-Collagen (porcine collagen), D-Col (denatured collagen), Technology Lipido-Colloid (TLC) Nano Oligosaccharide Factor (NOSF), a synthetic dressing with prevalence on the European market and a non-woven control dressing. Thirty-six diabetic (db/db) female mice received two full-thickness excisional wounds. Wounds were treated with a pre-moistened control or dressing (12 wounds per group). Dressings were changed or re-applied after 3 days according to the products' instructions. After 7 days, macroscopic images were taken, and wounds harvested. Wounds were bisected and processed for histological and biochemical analysis. Image analysis was performed on Haematoxylin&Eosin stained (to measure wound length and granulation tissue) and immuno-stained slides to see the effect of dressings on inflammatory cells and re-epithelialisation.

RESULTS: Image analysis of stained sections showed that Col/ORC/Ag treated wounds achieved higher level of re-epithelialisation ($p < 0.0001$) compared to all treatment groups, while presenting larger granulation tissue than control ($p < 0.01$) and P-Col ($p < 0.05$) and inducing higher level of macrophages compared to the control ($p < 0.05$). Whereas Col/ORC and D-Col presented significantly more neutrophils than control ($p < 0.05$ and $p < 0.001$ respectively).

DISCUSSION & CONCLUSIONS: Preliminary results show that Col/ORC/silver achieves higher re-epithelialisation and modulates inflammatory cells to a higher degree than control. However, further in vitro analyses are needed to further elucidate these initial results. These results may help healthcare professionals with performing more choosing the best treatment for their patients.

ACKNOWLEDGEMENTS: The authors would like to thank the EPSRC grant reference EP/S022201/1 and 3M for funding this work.

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Keywords: Diabetic healing, In vivo and animal models

OP-154

Hydrophilic coated mucous penetrating nanoparticles for non – viral intestinal gene delivery

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INTRODUCTION: Gene delivery to intestinal mucosa would enable treatment of several debilitating disorders such as Inflammatory Bowel disease (IBS) effectively. Successful mucous-penetrating nanoparticles should carry contradictory surface properties to allow mucous diffusion and cellular uptake. Different strategies have been implemented to solve this charge dilemma including coating with hydrophilic polymers (1). In this project, cationic peptide nanoparticles of glycosaminoglycan binding enhanced transduction (GET) system for non-viral gene therapy were coated with hydrophilic co-polymer of hydroxyethyl acrylamide and 3-acrylamido propanoic acid (pHEA) to enhance the mucous penetration and achieve improved gene delivery to intestinal model.

METHODS: Nanoparticles were prepared by simple electrostatic interaction in solution and characterised for size and charge with DLS and encapsulation efficiency was estimated with YOPRO-1 assay. The transfection efficiency of the nanoparticles loaded with pDNA encoding GLuc reporter gene was tested in co-culture intestinal models using Caco2 and HT29-MTX cells. The mucous diffusion of the nanoparticles was investigated using stomach mucin suspension penetration across Transwells. in addition to diffusion across mucous producing cells.

RESULTS: Coating with pHEA generated negatively charged nanoparticles with size range 150-400nm and zetapotential (+16mV – -18mV) and > 90% of the loaded pDNA was encapsulated in the coated nanoparticles. Also the coating polymer protected the encapsulated pDNA against the activity of Dnase I enzyme. Modified nanoparticles generated 2-fold increased gene transfer compared to uncoated nanoparticles in the co-culture model. The mucous diffusion tested on Transwells showed similar diffusion of coated nanoparticles to other GET mucous penetrating formulations (40% PEGylated nanoparticles, (2)) with 47% relative nanoparticles diffusion after 4hrs. The confocal images of fluorescently labelled coated nanoparticles showed less entrapment within the mucous layer on the mucous producing cells compared to uncoated nanoparticles.

DISCUSSION & CONCLUSIONS: We show that the GET transfection system can be used for non – viral gene delivery in intestinal mucosal models, and coating with pHEA derivative generates negative nanoparticles capable of enhancing the transfection and mucous penetration. Employing pHEA polymer could be a valuable modification to enable gene therapy of mucosal tissues without the use of PEGylation.

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Keywords: Polymers - natural / synthetic / responsive, Nanomaterials (inc graphene)

OP-155

The use of Computer Vision for object detection in high throughput biomaterial discovery

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INTRODUCTION: High-throughput screening for biomaterials discovery is used when *ab initio* information is unavailable for rational design of new materials, generating data that reveals chemistries and topographies that control eukaryotic and prokaryotic cell behaviour. Unlike drug discovery screening where the liquid state means each chemical is separated in microwells, screening of solid materials requires microarrays/'chips' to present cells to candidate biomaterials. This often means no physical separation between each substrate, so segregation of cell response must occur in the analysis phase. This is typically quantitative analysis of brightfield and fluorescent images. Whilst fluorescent cell images are facile to segment automatically, biomaterial segmentation accomplished using imaging not dependent upon labelling (brightfield), which is less amenable to automated analysis due to lower contrast. Manual segmentation of biomaterials is possible but resource intensive for datasets with thousands of images. Here we propose a solution.

METHODS: Computer Vision is a field of computer science that replicates tasks humans do by sight, e.g. identify objects by shape. To assess this approach, different computational methods to automate biomaterial segmentation in brightfield images were trained using two types of screen: Polymer Microarrays and TopoChip. The former is a chemistry screen where each image (8,000+) contains a single feature with variable size/morphology; the latter a topography screen where each image (2000+) contains distinct, defined, repeating shapes. Two methods were compared: an open-source Region-Based Convolutional Neural Network tool for object detection (*detectron2*) and an algorithm approach using *OpenCV*. These were compared to manual segmentation using *ImageJ*.

RESULTS: For polymer microarrays, variability in material appearance meant *detectron2* could be trained to identify polymers. The precise, repeating nature of TopoChip features lacked sufficient variation to train a neural network. Therefore, an *OpenCV* algorithm using edge detection and template matching was developed that extracted topographies from the images. Both approaches segmented biomaterial features with over a 30-fold reduction in time versus manual processing.

DISCUSSION & CONCLUSIONS: We believe this is the first time Computer Vision is used to aid biomaterial discovery, demonstrating two approaches that can be implemented depending on the variability in appearance of the biomaterial resulting from manufacture by stochastic or precise techniques. Incorporation of automated object detection facilitates swifter, more robust analyses of image datasets by facilitating brightfield image segmentation, allowing additional analyses and reducing false positives/negatives. Segmenting biomaterials were converted to binary masks that improved subsequent fluorescent cell segmentation by allowing fore- and background

determination.

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Keywords: Imaging - advanced, Biomaterials

OP-156

Three-dimensional *in vitro* spheroid-based lymphangiogenesis model using click-crosslinked gelatin hydrogels

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INTRODUCTION: The lymphatic system plays a vital role in maintaining tissue fluid homeostasis and immune surveillance. Recent studies have uncovered the importance of vascular endothelial growth factor C (VEGF-C) in stimulating lymphangiogenesis for accelerating wound healing and resolving inflammation in chronic inflammatory diseases. As opposed to angiogenesis, lymphangiogenesis is often overlooked when designing *in vitro* models. Also, most studies use 2D monolayer cell culture, which do not accurately mimic complex vascular geometry. In contrast, 3D cell culture models can replicate the tissue microenvironment and cellular responses by enhancing cell–extra cellular matrix (ECM) interactions. Herein, we establish a 3D *in vitro* lymphatic endothelial cell (LEC) spheroid-based assay using gelatin hydrogels formed by bioorthogonal click-crosslinking [1], and show its suitability for studying VEGF-C-dependent lymphangiogenesis.

METHODS: Gelatin hydrogels (6, 8, 10 and 12% w/v) were characterised by their physicochemical properties using compression and rheological tests, and cytocompatibility using LIVE/DEAD and AlamarBlue assays. Transcript levels of selected LEC genes were evaluated in response to cell encapsulation using real time quantitative polymerase chain reaction (RT-qPCR). Inhibition of the transcription factor ERG in LEC was carried out using Lipofectamine transfection reagent and 20 nM siRNA oligonucleotides. To model lymphangiogenesis, LEC spheroids were embedded in hydrogels in the presence of VEGF-C (0 – 200 ng/mL) for 24 hr. LEC sprout number and length were quantified using ImageJ.

RESULTS: Gelatin hydrogels (6 – 12%) demonstrated tuneable mechanical properties ($E = 1 - 6$ kPa), gelation times (15 - 2 minutes) and biodegradability (24 – 96 hours), respectively. The viability and metabolic activity of LECs were preserved at gelatin concentrations < 12%. An inhibitory influence of increased matrix stiffness on LEC morphogenesis and marker genes (PROX1, LYVE1 and VEGFR3) was observed at high gelatin concentrations (> 8%). The lymphangiogenic activity of LEC spheroids embedded in 6% gelatin hydrogels showed a VEGF-C dose-dependent increase in sprout number and length. Interestingly, inhibition of ERG, an endothelial transcription factor required for VEGF-dependent angiogenesis, significantly decreased lymphangiogenesis, even in the presence of VEGF-C.

DISCUSSION & CONCLUSIONS: We have successfully established a mechanically tuneable 3D *in vitro* lymphangiogenesis assay using synthetic ECM-mimicking hydrogels. This can facilitate the investigation of lymphangiogenic modulating compounds and impact of gene expression on lymphangiogenesis. Ongoing studies are investigating the utility of this 3D model to study LEC mechanotransduction of ECM stretching and its role in promoting lymphangiogenesis.

ACKNOWLEDGEMENTS: Qatar National Research Fund (GSRA8-I-1-0210-2100)

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Keywords: Hydrogels and injectable systems, Vascular systems / vascularisation and heart

OP-157

Differentiation of human induced pluripotent stem cells towards notochordal-like cells: the role of tissue source

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INTRODUCTION: Notochordal cells (NCs) are linked to a healthy intervertebral disc (IVD), and they are considered an exciting target for cell-based therapy. However, NCs are scarcely available as they are lost early in life, and attempts at *in vivo* expansion have failed because NCs lose their specific phenotype. The production of Notochordal-like cells (NLCs) from human induced pluripotent stem cells (iPSCs) is a viable alternative. However, current attempts have been challenged by the low differentiation efficiency into the NC lineage. Therefore, the aim of this study was to build on the tissue-specific epigenetic memory of hiPSCs derived from IVD progenitor cells (TIE2⁺-cells) to improve hiPSC differentiation towards mature, matrix-producing NLCs.

METHODS: hiPSCs were generated from TIE2⁺ cells of three adult donors. As a comparison, donor-matched minimally invasive peripheral blood mononuclear (PBM) cell-derived iPSCs were used. Firstly, the iPSCs were differentiated into mesendodermal progenitors by Wnt pathway activation (N2B27 medium + 3μM CHIR99021)¹. Thereafter, the cells were further driven towards the NC-lineage by transfection with synthetic *NOTO* mRNA¹ and further matured using a 3D pellet culture in discogenic medium containing 10ng/mL TGF-β1. Read-out parameters included cell morphology, gene and protein expression and matrix deposition.

RESULTS: Both TIE2⁺ and PBM cell-derived hiPSC showed successful differentiation towards mesendodermal progenitor cells following Wnt activation on day 2, indicated by the cells moving out of the colonies after CHIR stimulation. Accordingly, a decreased gene expression of pluripotency markers (*OCT4*, *SOX2*, *NANOG*), and upregulation of Wnt-target genes (*LEF1*, *NODAL*) and mesendodermal markers (*TBXT*, *FOXA2*, *TBX6*) was observed compared to mTESR1 controls. This was confirmed by immuno-stains for FOXA2 and TBXT. At day 3, we confirmed a 9-fold increase in *NOTO* mRNA levels after transfection in all donor lines. At day 28, the appearance of vacuolated NLCs was observed in both TIE2⁺ and PBM cell-derived pellet cultures confirming successful commitment towards the NC-lineage. Interestingly, while DMMB-assay detected GAG deposition in both lines, a significant increase in GAG content was seen in the TIE2⁺ cell-derived pellets.

DISCUSSION & CONCLUSIONS: Tissue-specific TIE2⁺ cell-derived iPSCs may allow for an improved iPS-NLC differentiation efficiency, indicated by the increased potency for deposition of GAG-rich matrix. Detailed analysis of the phenotypic markers and matrix deposited at the end of the 28 day maturation is ongoing to further document the phenotype of these iPS-NLCs. Delineating which epigenetic features are retained after reprogramming of these two cell lines, could shed light on the differences in their differentiation capacity.

REFERENCES: ¹Colombier *et al.*, 2020

Keywords: Induced pluripotent stem cells, Intervertebral disc / spine and their disorders

OP-158

Combining physical properties of collagen/hyaluronan hydrogels and chemical stimulation with growth factors promotes mesenchymal stem cells differentiation into NP cells

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INTRODUCTION: Low back pain is often associated with intervertebral disc (IVD) degeneration (40% of cases). Beside surgery, novel treatments relying on stem cells injection into Nucleus Pulposus (NP) were tested to promote IVD regeneration. Unfortunately, the outcomes were disappointing because of cell leakage and incomplete differentiation. Nowadays, a consensus exists on the necessity to encapsulate stem cells within a hydrogel to in situ maintain them and favor their differentiation. As cell behavior depends on biochemical and physical environment, a biomimetic hydrogel would promote IVD regeneration.

We recently developed collagen/ hyaluronic acid hydrogels (1:5 ratio) possessing mechanical properties, structure and hydration mimicking the physical properties of Nucleus Pulposus. In this study, we hypothesized that mesenchymal stem cells encapsulated within these biomimetic hydrogels would differentiate into NP like cells to promote IVD regeneration. We also evaluated the effect of GDF-5 and TGFβ1 (two NP differentiation factors), as factors potentializing the physical stimuli.

METHODS: Hyaluronic acid (HA) was functionalized with tyramine groups (HA-Tyr) and mixed with type I collagen using the 1:5 collagen/HA-Tyr ratio (collagen:0.4%, HA-Tyr 2%). Then, human bone mesenchymal stem cells (BM-MSCs) were added and hydrogels formed. These biomimetic hydrogels were cultivated over 28 days with 10% fetal bovine serum (proliferation medium) or with GDF5 and TGFβ1 (differentiation medium). Pure HA-Tyr and collagen hydrogels were used as controls.

RESULTS: The composite collagen/HA-Tyr hydrogels were stable over 28 days. On the opposite, the pure collagen hydrogels drastically contracted and the HA-Tyr ones swelled. Cell viability within pure collagen hydrogels was high but MSCs did not proliferate. In composite and pure HA-Tyr hydrogels, cell adhesion was lower but cells proliferate (population X4 at day 28). The mechanical properties measured in collagen/HA-Tyr hydrogels were higher than those in pure collagen and HA-Tyr hydrogels. In composite hydrogels, cells exhibited a rounded morphology similar to NP cells. The differentiation medium did not impact cell viability, mechanical properties and cell morphology. The phenotypic analysis revealed that a high HA-Tyr content within hydrogels promoted the gene expression of collagen II, Sox 9 and aggrecan, three NP markers and inhibited IBSP and collagen I, osteoblast and fibroblast markers. The differentiation medium potentialized the gene expression of NP markers as it appeared sooner and was higher.

DISCUSSION & CONCLUSIONS: Taken together, these results show that hydrogel properties and growth factors (GDF5 and TGFβ1) act in synergy to promote the differentiation of BM-MSCs into NP cells. Thus, these hydrogels are promising for IVD regeneration.

Keywords: Multipotent (mesenchymal) stem cells, Intervertebral disc / spine and their disorders

OP-159

3D Bioprinting Whole Intervertebral Discs to Understand Development & Inform Regenerative Therapies

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INTRODUCTION: Back pain is a leading cause of disability worldwide, affecting an estimated 632 million people, with intervertebral disc (IVD) degeneration being implicated in ~40% of cases. As treatment options are limited, novel IVD disease models and regenerative approaches are essential. Thus, this project uses 3D bioprinting technology to replicate the structural, mechanical, and biochemical features of different regions of the IVD to investigate the drivers of disc development and degeneration and inform regenerative therapies.

METHODS: A bioprintable IVD was designed using BioCAD (RegenHU), complete with disorganised nucleus pulposus (NP) and aligned annulus fibrosus (AF) regions. Human NP and AF cells were seeded within shear-thinning crosslinkable alginate-collagen (AlgCol) and alginate-collagen-laminin (AlgCollam) hydrogels and a biphasic IVD model bioprinted using suspended layer additive manufacturing (SLAM), a novel technique for patterning low-stiffness (<100kPa) materials. The bioprinted IVDs were cultured in normoxia or hypoxia for 21 days and mechanically characterised using rheology. Cell tracker dyes were used to ascertain IVD stability and cell migration, whilst cell viability was verified using confocal microscopy. Tissue development was examined by using RNAscope technology and immunostaining for key IVD cell markers, including collagen types I & II and hyaluronic acid.

RESULTS: A range of IVD models with regionally distinct cell populations, stiffnesses, and compositions were created, with NP regions of physiologically relevant stiffnesses (0.5–5 kPa) and a stiffness gradient between NP and AF. Cell tracker studies indicated that the NP, AF and NP-AF interface were maintained over 21 days, whilst cell viability remained high throughout. Differences could be observed in the pattern of HA staining between different IVDs. Increased matrix stiffness significantly reduced the area and intensity of NP cell matrix production. Laminin did not significantly affect deposition but encouraged cell clustering. Hypoxia resulted in strong punctate staining relative to normoxia and an increase in HA intensity and integrated density.

DISCUSSION & CONCLUSIONS: To our knowledge, this represents the first presentation of a bioprinted whole IVD enabling the control of physical, biochemical, and structural matrix cues. The findings shed light on the importance of tissue stiffness and stiffness modulation in driving irreversible tissue degeneration. Work is ongoing to further elucidate the impact of stiffness, bioink composition, microenvironmental cues and regionalisation on ECM production.

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Keywords: 3D printing and bioprinting, Intervertebral disc / spine and their disorders

OP-160

Screening of Regenerative microRNAs in the Nucleus Pulposus of the Intervertebral Disc using Cell Penetrating Peptide Delivery

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INTRODUCTION: Lower back pain is a global social and epidemiological issue, affecting up to 80% of people throughout their lives, and is believed to be triggered by degeneration of the intervertebral disc [1]. Early-stage degeneration stems from a loss of glycosaminoglycans from the nucleus pulposus (NP) region, leading to reduced disc height, nerve compression, and pain [2]. microRNAs (miRs) are short, non-coding nucleic acids and are currently being explored for their therapeutic potential [3]. The objective of this work was to investigate the regenerative potential of miRs-140-5p, 149-5p, 141-3p inhibitor, and 221-3p-inhibitor which have been shown to elicit a positive response in disc and cartilage cells. To deliver these miRs, non-viral cell penetrating peptides (CPPs) including cationic 30mer amphipathic peptide (RALA) [4], and GAG-binding enhanced transduction FGF2B-LK15-8R (GET-FLR) [5] were investigated, with Lipofectamine RNAiMAX [6] serving as a positive control. **METHODS:** NP cells were enzymatically isolated from Wistar rats and culture expanded. Carrier-miR complexes were prepared at previously identified optimal charge ratios. Three days post-transfection, protein expression was quantified using immunofluorescence, with antibody staining for regenerative proteins: ACAN, COL2, and SOX9. Mean fluorescence intensity was quantified using CellProfiler 4.2.1, with normalisation to non-transfected groups. All experiments were carried out in triplicate, differences were assessed by one-way ANOVA with Tukey's multiple comparisons, p-values <0.05 were considered significant.

RESULTS: Following miR-140 and miR-141-inhibitor transfection, ACAN, COL2, and SOX9 protein expression was significantly upregulated. Transfection of miR-149 led to a significant increase in ACAN and SOX9. miR-221-inhibitor transfection enhanced protein expression of ACAN and COL2 to a significant degree. Protein expression was increased by approximately 1.5-fold across groups and changes were more evident following transfection with RALA and GET-FLR vectors when compared to Lipofectamine.

DISCUSSION & CONCLUSIONS: This work screened potential regenerative miRs in a rat 2D in vitro NP model, with increased protein expression observed following transfection. This workflow allows comparison of functionality and therapeutic capacity of miRs delivered with different CPPs, to identify miRs warranting further investigation in more physiologically relevant 3D and organ culture models.

ACKNOWLEDGEMENTS: This work was supported by the European Research Council (ERC-2019-CoG-864104; INTEGRATES).

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Keywords: Cell therapy, Gene therapy

OP-161

Phenotypic maturation of hiPSC-derived notochordal cells using covalent hydrogel and 3D culture

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INTRODUCTION: Low back pain is a worldwide health problem. Its onset is often associated with the degeneration of the nucleus pulposus (NP), the central part of the intervertebral disc (IVD). The founder cells of the NP originate from the embryonic structure called the notochord. After birth, the matured notochordal cells (NC) have been shown to exert rejuvenating effects upon degenerated IVD cells [1]. Human induced pluripotent stem cells (iPSC) differentiation toward notochordal-like cells (NLC) has been reported for cell-based therapeutic interventions to mediate regeneration. However, these NLC harbor a transcriptomic signature of immature embryonic NC [2]. Phenotypic maturation should result in functional NLC producing specialized matrix that exert anabolic effects on degenerated disc cells. Therefore, there is a need to create an optimized microenvironment suitable to sustain NLC survival and to support their maturation toward a secretory phenotype.

METHODS: In this context, we investigated the effect of different 3D culture models on NLC maturation and differentiation. Based on previously described protocol, iPSC were first differentiated into mesendoderm progenitors (MEPC) by WNT activation, and then, NLC differentiation was triggered by NOTO mRNA transfection [2]. The MEPC transfected or not with NOTO were either aggregated into micromass or encapsulated in HA-based hydrogels grafted with specific peptides. These 3D constructs were kept in culture in NLCs differentiation medium or in nucleopulpopogenic medium to study their maturation toward NP-like cell phenotype [3]. Cells' identity, proliferation and differentiation were assessed at different time points by qPCR, immunohistochemistry, or immunofluorescence for confocal imaging.

RESULTS: Our results demonstrated that the tested 3D culture cannot substitute the transfection of NOTO mRNA for the commitment of the MEPC toward notochordal lineage. Interestingly, when MEPC were encapsulated in hydrogels, we observed clusters of cells with notochordal identity and structures resembling cytoplasmic vacuoles suggesting that cells began their maturation. Following 28 days differentiation in nucleopulpopogenic medium, aggregated MEPC micromass showed a cell-dense part with positive staining for known NP markers including CA12, SOX9, CD166 and CK8 associated with a matrix-rich part composed of glycosaminoglycan proteins such as aggrecan.

DISCUSSION & CONCLUSIONS: This work demonstrates that 3D microenvironment is favourable for phenotypic maturation of the NLC. Analyses are ongoing to determine cells responses to different viscoelasticity properties and cell-matrix interactions.

ACKNOWLEDGEMENTS: Financial support from EU Horizon 2020, "iPSpine" and the French Society of Rheumatology, "Spherodisc".

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Keywords: Induced pluripotent stem cells, Differentiation

OP-162

Bulk RNA Next Generation Sequencing of Native Donor-matched Human Tie2+ Progenitors versus Tie2- Cells

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INTRODUCTION: Stem cell therapy of the degenerate and painful intervertebral disc (IVD) is one of the most warranted but also highly disputed procedures to be applied in patients with low back pain. A particular autochthonous progenitor cell population has been identified in the center of the IVD, so-called nucleus pulposus (NP) progenitor cells (NPPC), positive for angiopoietin-1 receptor (= CD202b, Tie2, TEK gene). Here, we present novel Next Generation Sequencing (NGS) data and compare the transcriptome of FACS-sorted Tie2+ NPPC with Tie2- IVD cells and unsorted native cells. Furthermore, we explored whether expansion in presence of bFGF2 influenced the transcriptome.

METHODS: Primary cells were isolated from N = 32 donors with written ethical consent of the Insel University hospital (Swissethics # 2019-00097). Inclusion criteria were 18-70 years of age, males and females alike undergoing spinal surgery. Cells were isolated from trauma tissue by 1h pronase followed by collagenase 2 digestion overnight. The cells were then expanded on plastics in normoxia and low-glucose DMEM + 10% FCS ± 2.5µg/mL bFGF-2. When passage 0 was 90% confluent, the cells were trypsinized, labelled using Tie2+ antibody, and then sorted using a FACSARIAIII. For bulk RNA-sequencing we isolated 17 high quality total RNAs at passage 2 Tie2± cells and from passage 1 unsorted donor-matched cells from 5 donors using a column purification method. All RNA was checked for integrity RIN > 7.0 and absence of contaminants. RNA sequencing was performed at the Functional Genomics Center Zürich. Extracted RNA was prepared for sequencing using the Illumina TruSeq Stranded mRNA Library Prep assay.

RESULTS: There was no significant correlation between donor age and the percentage of NPPC Tie2+ cells in 1st passaged cell populations (N = 32 patients). In two donors, no Tie2+ cells could be observed. Transcriptomics showed distinct clustering of samples that were either Tie2+ sorted, Tie2- or unsorted. Tie2+ selected cells revealed 551 differentially expressed genes (DEGs) (p < 0.001) compared to Tie2- cells; KRT19, a previously described marker for “nucleopulpopocytes”, was significantly upregulated in Tie2+ cells; ACAN, PEG3, IGF2BP3, SERPIN1 and PRG4 (lubricin) were significantly down-regulated. GO Pathway analysis showed an enrichment of matrix genes and genes involved in cell cycle control and matrix production.

DISCUSSION & CONCLUSIONS: Here, new “molecular markers” for Tie2+ cells were identified.

Furthermore, the gene expression profile of Tie2+cells pointed to a reduced expression of ECM markers but increased stem cell markers.

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Keywords: Stem cell niche, Cell therapy

OP-163

Sulfated hydrogels as primary intervertebral disc cell culture systems

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INTRODUCTION: Intervertebral disc (IVD) degeneration is a key contributor for low back pain, a leading cause of disability worldwide¹. During degeneration, IVD aging is accelerated, leading to progressive structural changes, including blood vessel and nerve ingrowth that promote discogenic pain². In vitro studies require novel biomaterials that mimic the IVD extracellular matrix (ECM) to provide mechanical support and a reservoir of cytokines and growth factors. As proteoglycans with their attached sulfated glycosaminoglycans (GAGs) are one of the major components of the ECM, the ECM's sulfation state could be a key factor for IVD cell-fate³. Thus, we aim to explore human NP cell fate using a novel sulfated alginate model with varying degrees of sulfation (DS).

METHODS: Primary human NP cells were expanded, mixed with solutions of i) 2.5% of standard alginate, ii) 0.1 DS, and iii) 0.2 DS alginate (4 x 10⁶ cells/ml) and casted in 27 µl cylindrical-shaped carriers (4 mm diameter, 2 mm height). Carriers were cultured for two weeks for phenotype recovery and were collected with the culture media on day 0, 7 and 14.

RESULTS: A significant decrease of cell density ($p < 0.05$) was observed in 0.2 DS alginate after 7 and 14 days of culture. Similarly, cell viability was significantly reduced ($p < 0.05$) in 0.2 DS alginate after 7 days of culture (N=4). In addition, cell metabolic activity tended to be decreased in 0.2 DS alginate compared to standard alginate after 14 days of culture. Surprisingly, ECM remodeling factors such as MMP2 and TIMP1 were slightly upregulated in the 0.1 DS group (N=1), whereas catabolic cytokines were downregulated in the 0.1% DS group.

DISCUSSION & CONCLUSIONS: We demonstrate significant cellular differences between 0.2 DS alginate vs standard alginate and 0.1 DS alginate. Particularly, a significant decrease in cell density, metabolic activity and viability were observed in the 0.2 DS alginate after 7 days of culture. According to the secretome, the sulfated alginate group seems to possess increased catabolic ECM remodeling with lower secretion of catabolic factors, suggesting less responsive NP cells to ECM structural changes. Overall, standard alginate seems to be the best option for NP cell 3D culture models.

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Keywords: Hydrogels and injectable systems, In vitro microenvironments

OP-164

Bioinspired Hydrogels Promote Tenogenic Stem Cell Commitment by Synergistic Control over Topographical Cues and Growth Factor Signaling

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INTRODUCTION: Tendon diseases are prevalent health concerns for which current therapies present limited success. Tissue engineering approaches are attracting growing interest to enhance the intrinsically low regenerative capacity of tendons. In this context, a concomitant modulation of both biophysical and biochemical stimuli is hypothesized to improve the tenogenic commitment of stem cells, thereby promoting regeneration. We have previously shown that molecularly imprinted nanoparticles (MINPs) can function as artificial receptors for endogenously secreted growth factors (GFs), boosting their paracrine activity.¹ Here, MINPs are combined with bioinspired anisotropic hydrogels² to manufacture 3D tenogenic constructs.

METHODS: MINPs were solid phase-imprinted using a TGF- β 3 epitope as template. Their affinity for the target was assessed by surface plasmon resonance and dot blot. Magnetically-responsive microfibers were produced by cryosectioning electrospun meshes containing iron oxide nanoparticles. Tenogenic constructs were prepared by encapsulating adipose tissue-derived stem cells (ASCs), along with microfibers and MINPs, in enzymatically-crosslinked gelatin hydrogels. Microfibers were unidirectionally aligned by applying a uniform magnetostatic field during gelation. Cell responses were analyzed by multiplex immunoassay, quantitative polymerase chain reaction (qPCR), and immunocytochemistry.

RESULTS: MINPs showed a notable affinity for the template (18 ± 13 nM), comparable to monoclonal antibodies. Encapsulated ASCs acquired an elongated shape and predominant orientation corresponding to the alignment axis. Multiplex immunoassay revealed increased TGF- β signaling via non-canonical Akt/ERK pathways when MINPs were combined with aligned microfibers. qPCR findings demonstrated an upregulation of tendon-associated gene expression in association with MINP concentration in aligned constructs, in contrast to randomly oriented gels. Immunostaining of tendon-related proteins (scleraxis, tenascin-C) also presented similar outcomes, corroborating the hypothesis of phenotypic driving toward tenogenesis.

DISCUSSION & CONCLUSIONS: Our results demonstrate that microstructural cues and biological signals synergistically steer ASC fate commitment toward tenogenesis. The GF-sequestering ability of MINPs allows a cost-effective replacement of recombinant GFs, potentially decreasing the translational costs of tissue engineering strategies. Simultaneously, magnetic microfibers permit an efficient modulation of topography within hydrogels, mimicking native tissue architecture. The

integration of both components thus appears critical for a robust direction of stem cell differentiation. Hence, this strategy holds potential to significantly improve tendon healing while also being adaptable to engineer other tissues.

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Keywords: Composite materials, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-165

Cell-scale pores in microporous annealed particle (MAP) hydrogel implants promote regeneration in volumetric muscle loss injuries

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INTRODUCTION: Skeletal muscle possesses high regenerative capacity, but severe injuries can cause volumetric muscle loss (VML), or irrecoverable loss of muscle tissue and function. VML injuries are prone to fibrosis due to acute inflammation and loss of supporting structures. No effective treatments are clinically available; however, hydrogels are seen as potential material approach due to their physical resemblance to muscle. Microporous annealed particle (MAP) hydrogels are a new subclass of hydrogels composed of an injectable slurry of microscale hydrogel spheres that are transitioned on demand (i.e., annealed) into a scaffold with interconnected, cell-scale pores. Using MAP hydrogel, we observed improved regeneration compared to a solid nanoporous hydrogel in a rodent VML model.

METHODS: We made MAP hydrogel consisting of polyethylene glycol backbone polymer, an enzymatically-degradable peptide crosslinker, and a light-based annealing moiety. The same formulation was used to create nanoporous hydrogels. We used a model of VML in rats, wherein ~20% of the tibialis anterior (TA) is surgically removed. Our treatment groups, injected within the defect following its creation, were MAP hydrogel, nanoporous hydrogel, and sham. We had two time points (4 and 12 weeks post-op) for muscle harvest and histology.

RESULTS: Muscles treated with MAP had a higher ratio of pro-regenerative macrophages (CD68+/CD163+) than those treated with nanoporous hydrogel at 4 weeks ($p = 0.0092$). At 12 weeks, the trend was maintained, but lost significance (MAP: $53.7 \pm 12.9\%$, nanoporous: $42.2 \pm 9.5\%$). Muscles treated with MAP did not have a detectable fibrotic capsule at any time point, while those treated with nanoporous gel had fibrotic capsules at 4 weeks (221.7 ± 119.6 μm thick) and 12 weeks (35.8 ± 20.5 μm thick). Interestingly, new muscle fibers were detected by picosirius red and myosin heavy chain immunostaining within the MAP implants at 4 and 12 weeks, while none were inside the nanoporous implants at either timepoint.

DISCUSSION & CONCLUSIONS: The new muscle fibers within the MAP implant and absence of a fibrotic capsule indicate that cell-scale porosity allow cells to quickly infiltrate and differentiate, which is hindered by the fibrotic capsules that form around nanoporous implants. Macrophages are known to play an important role in muscle repair and MAP was able to shift their polarization relative to the nanoporous control. We conclude that the MAP hydrogel platform is a promising approach for VML and can improve muscle regeneration over the traditional solid nanoporous approach to hydrogels.

Keywords: Hydrogels and injectable systems, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-166

Mimicking cell-matrix crosstalk: bioinspired gelatin hydrogels functionalized with monomeric fibronectin as a liver *in vitro* model

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INTRODUCTION: Current liver *in vitro* models show many limitations and low similarity with the organ as they do not recapitulate the 3D *in vivo* microstructure and composition. Therefore, the design of powerful three-dimensional (3D) models that mimic the liver matrix and cell-matrix interactions remains to be explored to finally unseat 2D cultures. This work aims to design a new 3D culture platform for hepatic cell based on gelatin (GEL) hydrogels functionalized with covalently bonded monomeric fibronectin (FN). The composition is inspired by the natural matrix: GEL (denature collagen) representing collagen as the main component and FN as a relevant adhesion molecule in cell-matrix communication.

METHODS: GEL hydrogels cross-linked with a 4-arm-polyethylene-glycol (PEG) were synthesized at 3 different GEL:PEG %w/v ratios (1:1, 1:0.75 and 1:0.5). Their mechanical properties and gelation times were determined by rheology and equilibrium water content was also calculated. Monomeric FN, previously denatured, was incorporated in hydrogels and the retention was assessed by ELISA. Finally, HepG2 cells were encapsulated in hydrogels with and without FN, in matrigel and monolayer. The effect of 3D culture on liver functionality was tested, as well as the effect of FN. For this purpose, LIVE/DEAD assay, albumin and urea production were evaluated. In addition to this, changes in gene expression of key hepatic markers were tested by qPCR.

RESULTS: Hydrogels showed tailored mechanical properties and fast gelation time (approximately 2min). The ratio 1:0.75 displayed storage modulus values near the native tissue (600Pa). This was the selected ratio to anchor the FN, which did not show changes in its mechanical properties after functionalizing. Release assay revealed that FN is effectively retained within the hydrogel (97%). HepG2 cells were alive after several days (over 90% of viability). Urea production increased significantly in cells cultured in GELPEG hydrogels with FN compared with cells in 2D and matrigel, indicating a positive effect of FN. Moreover, gene expression revealed significant advantages compared to monolayer cultures. The results demonstrated that FN presence enhanced the liver functionality of encapsulated cells.

DISCUSSION & CONCLUSIONS: In conclusion, these hydrogels could be considered as a 3D liver culture platform with significant potential to be explored for different applications such as drug screening or disease modelling as a replacement for conventional 2D models.

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Keywords: Biomaterials, Hydrogels and injectable systems

OP-167

High Throughput Platform for Understanding Macromolecular Interactions with Collagen and Hyaluronic Acid

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INTRODUCTION: The emergence of biopharmaceuticals resulted in a need for advancement in administration routes of the molecules. Subcutaneous administration, while advantageous, poses challenges concerning bioavailability of the molecules due to the complex transport process. The transport of the injected therapeutic is dictated by physiochemical properties of the molecules such as hydrodynamic radius, net charge and hydrophobic characteristics. The extracellular matrix (ECM) of the subcutis is largely composed of collagen, a fibrous protein, and hyaluronan (HA), an anionic polysaccharide. The high viscosity of HA is believed to act as a barrier for therapeutic transport. Currently, in-vitro, high-throughput platforms that allow transport screening through ECM models are limited.

METHODS: Transwell macromolecular recovery assay is a promising method that allows for systematic evaluation of the contribution of various ECM components on the transport of macromolecules. The method was first developed in [1-3]. Here, we utilize the Transwell assay with collagen, HA and combined collagen-hyaluronan (ColHA) hydrogels as in-vitro tissue models to determine mass transport properties of label-free macromolecules. Percent recovery of all molecules was measured at five time points. Recovery rates, defined as the slopes of percent recovery data, were calculated.

RESULTS: In this work, we demonstrate that the transport within collagen hydrogels is governed by steric interactions, with the macromolecular recovery being inversely related to the hydrodynamic radius of the macromolecules tested. Molecular recovery through HA solution is a function of both molecular charge and size. Negatively-charged macromolecules such as myoglobin had an increased recovery compared to lysozyme, a positively charged enzyme of similar hydrodynamic radius, which likely experiences attractive forces with the negatively charged HA. Size effects persist, as negatively charged bovine serum albumin recovery was lower compared to the less negative but smaller myoglobin. To assess the contributions of both collagen and HA simultaneously, hydrogels composed of collagen and HA (ColHA) were tested. Increasing HA content from 0 to 8 mg/mL within the collagen gels preserves the trends observed in collagen-only hydrogels with decreased the mass recovery of the macromolecules. This decrease in mass recovery is mainly the result of increased viscosity. Limited charge effects have been observed at high HA concentrations within the ColHA hydrogels.

DISCUSSION & CONCLUSIONS: Our results demonstrate that the Transwell platform can be utilized to screen molecular interactions and transport properties within tissue models.

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Keywords: Biomaterials, In vitro microenvironments

OP-168

Stimuli-responsive Cell-adhesive Micromaterials for Steering Stem Cell Fate within 3D Living Composite Tissues

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INTRODUCTION: Engineered living microtissues such as organoids have enormous potential for the study and regeneration of tissues and organs.[1] These models are typically formed via self-assembly of cells via cellular adhesion.[2] Consequently, these microtissues are material-free, and offer little to no mechanical control over their local microenvironment,[3] which has limited control over their behavior. Here, we report on the development of stimuli-responsive cell-adhesive micromaterials (SCMs) that can self-assemble with cells into 3D composite microtissues via integrin binding. We demonstrate that SCMs homogeneously distribute within microtissues and possess on-demand in situ tunable biophysical and biochemical properties, which enables cell programming via cell-material interactions. Specifically, cell behavior can be controlled based on the size, stiffness, number ratio, and biofunctionalization of SCMs in a temporal manner via orthogonal secondary crosslinking strategies.

METHODS: SCM production: Hydrogel precursor droplets composed of Dextran-Tyramine-Biotin (DexTAB) and horseradish peroxidase in phosphate buffered saline were emulsified in Pico-Surf 1 containing Novec 7500 Engineered Fluid using a microfluidic droplet generator.[4, 5]

Modular tissue engineering: RGD-functionalized SCMs were homogeneously co-seeded with cells into non-adherent agarose microwell chips.

In situ biochemical tuning: SCM-laden constructs were sequentially endowed with neutravidin, washed, incubated with biotinylated or desthiobiotinylated molecule-of-interest, and washed again.[6]

In situ stiffening: SCMs within modular tissues were in situ stiffened by incubating them with sodium persulfate and ruthenium. Free radical crosslinking was induced using 60 seconds of visible light irradiation. Lineage commitment was analyzed using histochemical staining.

RESULTS: Microgel droplets were formed in a microfluidic droplet generator and crosslinked by hydrogen peroxide supplementation. This resulted in formation of monodisperse DexTAB SCMs of tunable stiffness.

Next, the biotins in the SCM shell were functionalized with c(RGDfK), which enabled the self-assembly of SCMs within microaggregates owing to cell-microgel interactions.

In situ biochemical control was shown by sequentially endowing SCMs with (desthio)biotinylated molecules (i.e. fluorophores or antibodies).

Cell-microgel and cell-cell interactions within the microaggregate were optimized by controlling the amount, stiffness and/or size of the SCMs within the construct.

Moreover, photo-induced crosslinking allowed for on-demand control of SCM stiffness. Stem cell fate towards osteogenic and adipogenic lineages was temporally steered by in situ tuning the SCM stiffness within modular tissues.

DISCUSSION & CONCLUSIONS: In conclusion, we developed the first biochemically, biophysically, and spatiotemporally controlled SCMs for modular tissue engineering. This allowed for the creation of highly tunable and defined cellular microenvironments, which more accurately resembled the

dynamic microenvironment of cells in native tissues.

Keywords: Biomaterials, Hydrogels and injectable systems

OP-169

Highly tunable polysaccharide-based hydrogels to recreate cell microenvironments

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INTRODUCTION: Cells are embedded in a highly dynamic and complex surrounding. Synthetic hydrogels can recapitulate these environments to make tissue models and help us understand biological processes. We here describe a printable, photo-crosslinkable hydrogel system with tunable stiffness, degradation and presentation of binding sites to

METHODS: Alginate was chemically functionalized with norbornene groups, and characterized via ¹H-NMR and GPC. Hydrogels were formed by mixing the polymer with PEG or MMP-cleavable peptide flanked by two thiol groups and photoinitiator LAP, and shining 365 nm UV light for 30 seconds (10 mW/cm²). Mechanical properties were analyzed via rheology. The pre-gel solution was extruded through a 22G needle, pressure of 50 kPa and printing speed of 3 mm/s, using a Cellink. Printing fidelity was assessed by quantifying the spreading ratio (thickness of the deposited fiber divided by the needle's inner diameter). A variety of cells and organoids were encapsulated by mixing the cells with pre-gel solution, including thiolated-RGD. Cell-laden hydrogels were cultured and analyzed for viability and cell phenotype via immunostaining.

RESULTS: We obtained a range of biologically relevant mechanical properties of soft tissues, by altering polymer content and/or cross-linker concentration (323-2125 Pa). The viscous component before cross-linking was heavily influenced by initial polymer concentration.

4% alginate-norbornene pre-gel solution was successfully printable, with a spreading ratio of $2,26 \pm 0,24$. The deposited fibers had a diameter of $0,93 \pm 0,10$ mm.

Human endometrial organoids remained viable throughout the 7-day culture period. Dead cells were observed only as debris that did not have a circular or elliptical shape. Organoids with a healthy round shape did not contain dead cells. Organoids grew in size over time, and contained proliferative cells (ki67⁺), which expressed E-Cadherin showing cell-cell interactions.

Mouse embryonic intestinal organoids presented cells in proliferative state (ki67⁺), good cell-cell contacts (expression of E-cadherin), but remained as spherical organoids, with no branching, indicating an early not yet differentiated stage.

Thyroid follicles were highly viable and kept their GFP expression, indicating the maintenance of phenotype and non-differentiation into off-target cells. They presented a clustered morphology with inside lumens and production of Tg and T4 hormones.

DISCUSSION & CONCLUSIONS: We have developed a platform that allows the study of the influence of stiffness, matrix degradability and ECM binding motifs on a variety of soft tissue types, which opens the door to its use for disease modeling and patient-specific assays.

ACKNOWLEDGEMENTS: Funding: RegMedXB.

Keywords: Biomaterials, Stem cell niche

OP-170

Monitoring of Biohybrid Tissue-Engineered Vascular Prosthesis Remodeling by Hybrid 1H/19F MRI

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INTRODUCTION: Labeling scaffolds for 1H magnetic resonance imaging (MRI) poses challenges for detection, sensitivity, specificity, and quantification. These key aspects have increasingly motivated the investigation of 19F-MRI and new biochemical compounds. Indeed, novel imaging approaches combining hybrid imaging modalities and new materials with high content of highly mobile and chemically equivalent 19F atoms are highly required in routine clinical applications. Therefore, this study aims to enhance the monitoring of biohybrid tissue-engineered vascular grafts' (TEVG) remodeling via hybrid 1H/19F MRI.

METHODS: To enhance the visibility of our TEVG, filaments of highly fluorinated thermoplastic polyurethane (19F-TPU) were added to the textile scaffold, which was initially composed of non-degradable polyvinylidene fluoride monofilaments coated with biodegradable poly(lactic-co-glycolic acid) (PLGA) fibers labeled with superparamagnetic iron oxide nanoparticles (SPION), and moulded with fibrin gel, human endothelial and smooth muscle cells. First, the biocompatibility of the 19F-TPU fibers was assessed on both cell types via XTT. Then, a systematic investigation was carried out to determine the amount of 19F-TPU needed to generate a high signal-to-noise ratio via 1H/19F 7T MRI. Finally, the degradation of the biodegradable SPION-labeled PLGA coating was longitudinally assessed with and without cellular components.

RESULTS: The evaluation of cytotoxic effects on cells revealed the biocompatibility of 19F-TPU filaments. The superimposition of the grayscale T2W and "hot spot" ultrashort echo time (UTE) sequences of gelatin phantoms containing a different number of 19F-TPU fibers showed both enhanced visibility of the PLGA layer due to the SPION labeling and 19F signal intensity corresponding to a higher number of 19F-TPU fibers. The longitudinal monitoring of the SPION-labeled PLGA degradation and 19F-TPU fibers' permanent signal was carried out concomitantly. In both experimental setups, with and without cells, the R2 analyses showed a decrease in MRI signal after 7 days and 3 weeks, respectively, indicating hydrolysis of the PLGA, leading to SPION release. As postulated, no significant changes were observed regarding the non-degradable 19F-TPU signal intensity. Additional histological analyses supported the PLGA degradation and the constant presence of the 19F-TPU fibers.

DISCUSSION & CONCLUSIONS: Herein, we present a non-invasive imaging method to strengthen the investigation and resorption of vascular prostheses remodeling via hybrid imaging techniques. Our findings report the novel employment of innovative fluorinated and biocompatible MRI-traceable fibers, allowing the monitoring of both non- and biodegradable textile components of our TEVG. Thus, the presented approach might play a critical role in our TEVG theranostic and therapeutic application.

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Keywords: Polymers - natural / synthetic / responsive, Cardiovascular

OP-171

A miniaturised imaging window to guide and image foreign body reactions in vivo

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INTRODUCTION: The standardized assessment of the fibrotic reaction to the implantation of a biomaterial is based on ex vivo histopathological techniques that require a great number of sacrificed animals. Intravital microscopy allows instead to improve the resolution of microscopy images and to reduce the number of laboratory animals, but this procedure currently implies unethical and highly invasive imaging protocols. To advance the field, we developed a miniaturized imaging window, the Microatlas, integrating microscaffolds able to guide tissue regeneration in-vivo[1]. The Microatlas can be implanted subcutaneously in living organisms to allow tissue regeneration and time-lapse imaging at the interface with implanted biomaterials. By using label-free two-photon microscopy within Microatlas implanted in-vivo and repopulated by the host cells and blood vessels, here we quantified the foreign-body reaction in the same tissue district, at subsequent time points during implantation.

METHODS: We fabricated the micro scaffolds (500x500x100µm³) on glass coverslips (Ø12mm) by two-photon polymerization of a polymer called SZ2080. We performed the chorioallantoic membrane (CAM) assay in living chick embryos at 3, 4, 7 days after implantation of Microatlas. The imaging window was inspected in-vivo by two-photon label-free and confocal microscopy. Collagen-I was visualized in second harmonic generation (SHG). Embryos were sacrificed to perform ex-vivo inspections. The CAM cells were labelled with DRAQ5 and imaged by confocal microscopy or stained by Hematoxylin&Eosin paraffin-sectioned.

RESULTS: In chick embryos, confocal and two-photon inspections at implantation sites demonstrated growth of tissue inside the scaffolds and neo micro-vascularization with presence of a capillary density six times greater than in unimplanted control tissue. SHG showed the presence of a preferentially oriented layer of collagen-I, with a density comparable with control regions. Confocal microscopy allowed for quantification of cell density, that showed an infiltration rate two-fold greater than in untreated tissue. We identified and counted several infiltrated cell populations, including granulocytes, fibroblasts and endothelial cells.

DISCUSSION & CONCLUSIONS: The Microatlas was able to guide the host foreign body reaction to the microscaffolds, in terms of cell repopulation, collagen and capillary formation. This miniaturized device has the potential to be used as a reliable and ethical imaging window for intravital quantifications, potentially replacing the current highly invasive window chambers. With Microatlas we can reduce the number of animals employed in preclinical studies, refine, boost the analyses and replace expensive/lengthy analyses of cellular density, blood vessels sprouting, collagen and fatty infiltrates based on traditional histopathology.

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REFERENCES: 1.Conci et al, Adv.Opt.Mat.2022

Keywords: Imaging - in vivo, Imaging - advanced

OP-172

A new quantifiable approach in longitudinal intravital 3D microscopy reveals a response to injury feedback system between glomerulus and its renin cell niche

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INTRODUCTION: Renin cells (RC) reside in the juxtaglomerular apparatus and function as pluripotent progenitors capable of migrating into the glomerulus after injury. While regulation of these complex repair processes is poorly understood, the authors propose the hypothesis that glomerular repair processes in adults underlie an individual intraglomerular-juxtaglomerular feedback mechanism. Using longitudinal intravital microscopy and an injury model that induces local damage by targeted laser irradiation in individual glomeruli, enables observations of repair processes under controlled conditions. Using virtual ray tracing, an optical object characterization technique, a new method of quantification in longitudinal 3D intravital imaging, especially a detailed characterization of the renin cell niche, could be established.

METHODS: Eight-week-old female mRen-rtTAm2/LC-1/tdT renin reporter mice underwent doxycycline pulse induction and 7 days washout. Intravital microscopy was then performed through an implanted body window under an upright Leica SP8 multiphoton/OPO laser scanning microscope for three hours on up to seven consecutive days. Laser injury was induced by focusing 100% laser power for 5 seconds at 48x zoom on one Z-plane. 3D processing and analysis were performed with Bitplane Imaris 9.9.1 and novel 3D image quantification was realized by the Marching Cubes algorithm. Image stacks of glomeruli were used to calculate a surface model described by triangles. The calculation of the directional thickness of the renin cells in relation to a center was based on an adapted ray tracing method,

RESULTS: Targeted use of laser irradiation established an inducible, selective and reproducible intraglomerular injury model. Migrating RC extended during 3 hours towards the injury area, infiltrating the vicinity of this area without losing contact to the juxtaglomerular RC. During these processes the RC first increased in volume outside the glomerulus and then showed an up to threefold increased area of dynamic migration with up to 344% compared to their niche of origin area.

DISCUSSION & CONCLUSIONS: Longitudinal intravital microscopy combined with laser-induced intraglomerular injury and 3D image analysis of site-specific injury directed renin cell migration in transgenic mice was assessed in a quantifiable manner. The combination of these technologies provides a new powerful tool for directly studying the juxtaglomerular feedback system of renin cell recruitment/migration and transdifferentiation as a site-specific response to intraglomerular injury and may promise viable targets for further glomerular regeneration research.

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Keywords: Kidney, In vivo and animal models

OP-173

Advanced models for the study of meniscal tissue structure

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INTRODUCTION: The increased awareness of the significance of meniscus damage and associated joint degenerative changes, maximized by meniscectomy, recently led to increase efforts in developing regenerative and reparative approaches. However, the setup of advanced in vitro tissue models for the study and evaluation of the structural, biological, and mechanical characteristics of degenerated and treated menisci is still low, in particular considering the poor use of three-dimensional visualization techniques of the microstructure of the meniscus.

METHODS: In this study, a contrast-enhanced microtomography (CE- μ CT) approach – i.e. phosphotungstic acid (PTA) contrast, a specific agent binding to collagen fibres – has been investigated in order to increase tissue X-ray contrast, but still allowing histological processing. After formalin fixation, pars intermediae of human menisci retrieved from total knee replacements surgery were radially cut in four portions and randomly assigned to the following treatments i) contrast with 2% (w/v) PTA in 70% ethanol solution ii) contrast with 2% (w/v) PTA in water solution. For each group, samples were further divided by exposure times to the contrast agent i) overnight ii) 3 days iii) 7 days. μ CT scanning was performed after each scheduled experimental times for both PTA-contrasted groups. After imaging, a reversion process of the protocols to remove the PTA from the contrasted samples was performed to allow paraffin embedding and histological staining with Hematoxylin/Eosin, Picrosirius Red and Safranin O/Fast Green.

RESULTS: For both PTA contrast expositions, μ CT imaging showed a progressive diffusion of contrast from the periphery of the tissue progressively binding collagen fibres network in the inner parts, with superficial x-ray contrast at the earliest time up to a complete contrast for the samples exposed to 2% PTA in ethanol solution for 7 days. Histological staining of the same samples highlighted the preservation of tissue structure, allowing the evaluation of tissue's matrix and cellularity. The observation of birefringence pattern of collagen fibres with polarize light on the same cutting plane of μ CT images allowed a comparison and an evaluation of contrast diffusion according to presence of fibres in the sample.

DISCUSSION & CONCLUSIONS: Results suggest the possibility to integrate different two/three-dimensional visualization techniques of the microstructure of the meniscus, indispensable to deepen the knowledge about the relationship between tissue structure and function, mandatory to properly develop and validate advanced in vitro models.

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REFERENCES: Contrast Media Mol Imaging. 2019 Feb 28;2019:8617406. doi: 10.1155/2019/8617406

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Knee

OP-174

Construction and characterization of a multi-modal imageable biohybrid tissue-engineered heart valve

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INTRODUCTION: Valvular diseases often demand valve replacement, and the clinically available heart valve prostheses have considerable limitations which must be addressed. Tissue-engineered heart valves (TEHV) are promising solutions for valvular illness because of their remodelling and regenerative capacity. The bioreactor plays a vital role in achieving the in vitro maturation of the heart valve, and to monitor this maturation, the bioreactor must be able to undergo non-invasive imaging modalities like Magnetic resonance imaging (MRI) and Ultrasound (US). We constructed a pneumatically operated bioreactor that is entirely metal-free and consists of an ultrasound window, enabling both MRI and Ultrasound monitoring.

METHODS: The bioreactor was digitally constructed in Inventor software and produced out of poly (methyl methacrylate). An air chamber was installed to make the bioreactor completely electronic and metal-free, and only polyamide screws were used for fastening. Elongated tubes were used to bring the pressure regulating and data acquisition system far from the magnetic field of MRI. The biohybrid heart valve was produced using superparamagnetic iron oxide nanoparticles (SPION)-labelled poly (ethylene terephthalate) textile scaffold along with fibrin hydrogel and human arterial smooth muscle cells. Gated MRI sequences and ultrasound were performed to locate the valve and to evaluate its functionality.

RESULTS: The bioreactor was found to be MRI-compatible without any imageable distortion and blooming artefacts given by the polyamide screws or, in general, by the primary material constituting the bioreactor. Additionally, no leakage or contamination was observed throughout the valves' conditioning and MRI/US monitoring. Using the gated MRI sequences, the dynamic movement of the HV by the pneumatical system could be monitored and evaluated. Following the MRI investigation, the HV underwent US investigation, i.e., flow dynamics, flow velocity, and shear stress.

DISCUSSION & CONCLUSIONS: We introduce a heart valve bioreactor with multi-modal imaging ability. The bioreactor offers a physiological condition for valve closing and opening and a suitable environment for tissue maturation.

Keywords: Bioreactors, Imaging - in vivo

OP-175

Understanding how mechanical forces shape the hypertrophic niche by exploiting a developmental engineering model of endochondral ossification

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INTRODUCTION: Endochondral ossification (EO) is guided by physical and biochemical factors, together contributing to coordinated chondro-osseous cellular transitions and matrix remodelling. The transition zone between cartilage and bone is defined by hypertrophic chondrocytes and unique ECM. Identifying how force governs cartilage-to-bone trans-differentiation in the hypertrophic niche is relevant for understanding morphogenesis, pathology like osteoarthritis (OA) and will be critical for repair therapies. Previous studies from our group suggest transitions through the growth plate in adolescent mice are mechano-sensitive,¹⁻² but the underlying mechanisms remain elusive. Here, we model mechano-biological integration in human EO in vitro, alongside in vivo studies.

METHODS: To complement our in vivo work and humanise the research, we are engineering a developmentally-inspired EO model using gelatin methacryloyl-based hydrogel. Human bone-marrow stem cells are seeded into GelMA (characterized by NMR) with a controlled gradient of heparin methacrylate-BMP-2.³ Upto 35 days culture supports chondro-osseous differentiation during which cyclic compressive loading is applied. Relative hypertrophy and mineralisation is characterized using qPCR, μ CT, IHC. Cells from Confetti-ACANCre mice are being used in this model for cell lineage tracing.

RESULTS: Our previous work suggested that genetic perturbation of primary cilia in chondrocytes or increases in physiological loading, impaired trans-differentiation of epiphyseal hypertrophic chondrocytes². In the disrupted regions this is associated with altered VEGF presentation, increased pSMAD2/3 activity and sustained SOX9 expression. Impaired epiphyseal dynamics in ciliary cKO are rescued by off-loading the limb, supporting the mechano-dependence of coordinated cartilage-bone phenotypic transitions. MSC-laden constructs showed tri-phasic (bone-hypertrophy-cartilage) differentiation, as validated by Runx2/Sox9 staining. qPCR revealed increased expression of SPP1, SP7, Runx2, Col1 indicating osteogenesis at one end, distinct expression of Col10 and Runx2 in the central region of cellular hypertrophy and Sox9 and PRG4 expression at the cartilage end. The impact of mechanics on this chondro-osseous continuum, are being currently assessed. Ongoing work involves incorporating cells from in vivo models (Adipose-derived Confetti MSCs) to identify force-regulated cell and molecular events governing trans-differentiation.

DISCUSSION & CONCLUSIONS: Preliminary data suggests the formation of a humanised osteochondral gradient recapitulating developmental processes, in vitro. It offers a tractable system for dissecting key mechanisms in skeletal maturation by exploiting tunable material stiffness, growth factor gradients and extrinsic mechanics; parameters difficult to segregate in vivo. Further, the model will be integrated with snRNA and spatial transcriptomics and lineage tracing, to study trans-differentiation and thus shed light on cellular transitions of the hypertrophic niche.

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Keywords: Cartilage / joint and arthritic conditions, Microenvironment and niche engineering

OP-176

Acidic pH inhibits the stiffness response of human bone-marrow mesenchymal stem cells (BM-MSCs)

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INTRODUCTION: BM-MSCs have been proposed as a cell-based therapy for musculoskeletal diseases, often through direct implantation into the degenerate tissue. The physiochemical microenvironment within degenerate musculoskeletal tissue is complex and includes relatively hostile physical (high/dynamic mechanical forces) and chemical (hypoxia, low glucose, low pH) factors, to which implanted cells must endure, integrate and respond. During musculoskeletal disease initiation and progression, mechanical properties of tissues change coincident with changes in chemical factors. For example, tissue stiffening, and increased acidity occur in parallel within degenerating / injured intervertebral discs, bones and cartilage. However, how cells perceive and integrate mechano-chemical signaling has received little attention. Here, we assessed the proteomic stiffness-response of human BM-MSCs cultured on type I collagen-coated polyacrylamide gels at extracellular pH(pHe) akin to healthy (pH 7.1), moderately (pH 6.8) and severely (pH 6.5) degenerate musculoskeletal tissues.

METHODS: Human BM-MSCs isolated from hip joints were expanded on tissue culture plastic and transferred to type I collagen coated polyacrylamide gels of different stiffness (2 and 25 KPa). Following 3 days of culture in medium at pHe 7.1,6.8,6.5, cells were trypsinised and processed for label-free mass spectrometry (Orbitrap Exploris™ 480). The peptide abundances were quantified and mapped to proteins using MaxQuant. Differential expression, gene ontology enrichment and reactome pathway analysis were used to assess the systemic response of BM-MSCs to substrate stiffness at different pHe.

RESULTS: At pH 7.1 and 6.8 BM-MSCs were stiffness-responsive, increasing proteins in pathways related to regulation of matrix and collagen synthesis and secretion. Surprisingly, proteins involved in metabolic pathways, including the citric acid(TCA) cycle and electron transfer chain / ATP synthesis were down regulated. Differential expression analysis revealed an increase in collagen types I and III, fibronectin and connective tissue growth factor. Acidic pHe (pH 6.5) altered the mechano-response of BM-MSCs with an inhibition of ECM protein synthesis, loss of GO terms related to ECM composition and a decrease in cell signaling pathways.

DISCUSSION & CONCLUSIONS: This study demonstrates an integrated workflow for robust and reproducible analysis of mass spectrometry data enabling both specific protein assessment and system-wide interpretation of a mechano-chemical response in BM-MSCs. The stiffness-response of BM-MSCs is pH-dependent, which has implications for regenerative medicine and tissue engineering. Further studies are required to elucidate the mechano-chemical pathways operating in these cells, which could lead to discovery of novel targets for cell and tissue therapies/engineering.

ACKNOWLEDGEMENTS: This project is supported by the Republic of Türkiye Ministry of National Education.

Keywords: Multipotent (mesenchymal) stem cells, Biomechanics / biophysical stimuli and mechanotransduction

OP-177

Conservation of Mechanosignaling Gene Circuits Across Multiple Chronic Diseases

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INTRODUCTION: Biomaterial properties of the extracellular matrix and local biomechanical stimuli promote homeostasis, influence disease progression, and serve as targets for disease intervention. These biophysical cues have attracted considerable attention in some disease areas, such as the cardiovascular system. However, we still lack insight gained by horizontally integrating studies of individual conditions. To address this gap, we systematically screened for molecular underpinnings of biophysical effects on cell signaling and identified the molecular circuits that mediate the mechanobiology of chronic diseases, using systems biology and custom computational approaches. We hypothesized that mechanosignaling-related genes and pathways may be shared across multiple chronic diseases, and relate to pathogenesis. Such conserved molecular circuits may also contribute to development of Multiple Chronic Conditions (MCC), present in 25% of patients today and rising. **METHODS:** XML-files obtained from PubMed using Python and EDirect were used to populate custom databases of detailed citation information. We systematically mined titles and abstracts of 18 topic-specific citation bases, encompassing 9 predominant chronic diseases (cancer, cardiovascular, diabetes, obesity, arthritis, Alzheimer's, chronic kidney disease, epilepsy, chronic obstructive pulmonary disease) and 9 pertinent biophysical cues (e.g., pressure, stiffness, shear stress, and more) for gene and protein mentions. We used statistical and bioinformatics analyses to identify linked gene sets and derive de novo predicted mechano-regulatory circuits. We further interrogated the molecular patterns by correlating them with Medical Subject Headings (NCBI) and pharmacology drug databases.

RESULTS: We identified the frequency of individual human genes and proteins studied within the datasets of several major chronic diseases and related mechanical cues. A core set of molecules were detected across the biophysics or chronic disease literature, annotated by custom citation databases we produce during our computational analysis. Most genes cited in the biophysics datasets have also been studied in the context of chronic conditions, suggesting tight coupling between the fields. Our analyses also identified cell functions and gene circuits linked to chronic diseases, biophysical cues, and the convergence between these topics. We show also that more than one-third of genes associated with mechanoresponses can be modulated by drugs that have already been clinically approved.

DISCUSSION & CONCLUSIONS: Systems biology approaches to integrate chronic disease knowledge with mechanobiology literature uncovered novel, data-driven and disease-relevant mechanosignaling molecular circuits. These mechanosignaling insights into multiple major chronic health conditions can be applied in future to improve design of effective regenerative medicine therapies and treat chronic

conditions, a major public and personal health burden.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Omics / bioinformatics and systems biology

OP-178

Analysis of human fibroblast and keratinocyte secreted factors through conditioned media assays and proteomics

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INTRODUCTION: Identifying the molecular crosstalk of tissue resident cells can be instrumental for improving skin biofabrication, which holds great promise for better tissue models and improvements to wound healing interventions. Keratinocytes and fibroblasts secrete factors across the dermoepidermal junction, including cytokines, chemokines, ECM molecules, proteases, and growth factors, that regulate cell proliferation, migration, and differentiation. Cell conditioned media (CM) contain bioactive molecules that promote wound healing, reduce inflammation, and improve tissue regeneration. Herein, we identify proteins present in CM from human keratinocytes (KCM) and fibroblasts (FCM) and examine the effect of CMs on cell migration and proliferation in vitro.

METHODS: Fibroblasts and keratinocytes were isolated from skin obtained from healthy patients undergoing routine plastic surgery under approval from the Swedish ethical review authority (no 2018/97/31). Conditioned media were collected in basal media (KSFM or DMEM) for each cell type, filtered through 3kDa filters to concentrate protein content, and quantified using BCA. Proliferation and migration were analyzed for each cell type in each CM, with and without supplementation, using scratch assays, cell counting, and the LiveCyte II kinetic cytometer. Protein content was analyzed using SDS-PAGE and LC-MS/MS (Q Exactive HF Hybrid Quadrupole Orbitrap). Proteomic analysis was done using SEQUEST HT, PANTHER, EnrichR, and STRING.

RESULTS: Fibroblasts and keratinocytes showed greater proliferation but lower migration in both CM compared to standard media, after three days. Both cell types showed the shortest population doubling time when cultured in CM. SDS-PAGE gave weak bands in CM corresponding to bands present in complete media. Proteins from serum content in fibroblast media overshadowed the fibroblast secretome. LC-MS/MS identified more peptides in KCM (559) than FCM (12) after filtering out *Bos taurus* hits. Common in both CM were 14-3-3 sigma, Serpin B7, IGFBP7, TIMP1, Cystatin-C, S100-A6, and EIF5A1. The ontology enrichment analysis found protein sets relevant to epidermis development, inflammation, ECM production, cell adhesion, metabolism, and apoptosis. Around 65% of proteins identified in both CM were annotated as extracellular.

DISCUSSION & CONCLUSIONS: Using the kinetic cytometer provides high content analysis instrumental in describing phenotypes and behaviours of cells in conditioned media. Identification of the CM proteome is a first step in delineating fibroblast-keratinocyte crosstalk. Repeating experiments by analyzing the proteomic response to CM will provide the next steps in understanding the role of co-regulation in tissue organization.

ACKNOWLEDGEMENTS: This study was funded by the Swedish state and Region Östergötland under the ALF-agreement (RÖ-969701).

Keywords: Wound healing, Skin

OP-179

Transplantation of autologous CFSE-labeled fibroblasts on gelatin microcarriers in a porcine wound-healing model

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INTRODUCTION: Wounds are a worldwide problem in healthcare, incurring major costs annually. Extensive wounds require skin autotransplantation, which saves lives, but can result in donor-site morbidity, suboptimal outcomes, and are time- and resource-demanding. An alternative intervention, involving autologous cells seeded on microcarriers, may lead to improved outcome and can have advantages over cultured epithelial autografts. Understanding the contribution of cells and scaffolds to the healing process requires techniques for discriminating transplanted cells from resident cells. Using an intracellular fluorescent dye, carboxyfluorescein succinimidyl ester (CFSE), we tracked transplanted cells to analyze cell viability, proliferation, and tissue incorporation.

METHODS: Autologous dermal fibroblasts were stained with CFSE, seeded on porous gelatin microcarriers (PGM), and transplanted to full-thickness porcine wounds on two female pigs, under approval from the regional animal ethics committee (ID 534). The experimental groups included wounds transplanted with PGM seeded with fibroblasts, PGM without cells, and non-transplanted control wounds. Wounded skin tissue was biopsied, cryosectioned, and analysed after three, seven, 14, and 28 days, post transplantation.

RESULTS: CFSE-stained fibroblasts could be detected within the tissue at all timepoints. Transplanted fibroblasts had migrated out from the PGM and into the granulation tissue after three days. The fluorescence intensity of CFSE decreased over time, and was generally redistributed into the stroma as well as the epidermis. CFSE+ Ki67+ cells were detectable by immunohistochemistry and could be associated to tissue structures stained with pancytokeratin and alpha-SMA. After 14 days, the epidermal thickness of the wounds receiving empty PGM, and PGM + Fibroblasts, was significantly thicker compared to both the non-transplanted wounds and to uninjured skin. By day 28, the epidermis of all groups had matured, and no difference could be seen compared to uninjured epidermis.

DISCUSSION & CONCLUSIONS: The current study shows that CFSE is a stable marker for transplanted cells in tissue and can be followed in vivo for 28 days. These experiments confirm that transplanted fibroblasts seeded on PGM actively migrate out of the microcarriers and into the dermis. It was also evident that the fluorescence intensity of CFSE decreased over time, partly due to cell proliferation. Immunostaining for Ki67 indicates that some transplanted CFSE+ cells remain proliferative in the tissue. Future studies will focus on transplanting co-cultures of fibroblasts and keratinocytes and evaluating quality of healing after transplantation.

ACKNOWLEDGEMENTS: This study was funded by the Swedish state and Region Östergötland under the ALF-agreement (RÖ-969701).

Keywords: Wound healing, Cell therapy

OP-180

Polydopamine functionalized hydrogel with nitric oxide release and photothermal effects for bacteria-infected wound healing

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INTRODUCTION: It is significant to develop alternative antibiotic treatments to combat the increasing number of multidrug-resistant bacteria. Photothermal therapy based on polydopamine (PDA) has the advantages of controllability, low invasiveness, and low possibility of causing MDR bacteria. However, the stability and anti-bacterial properties of PDA nanoparticles were insufficient to remove heat-resistant bacteria. Studies have shown that hydrogel can reduce the aggregation of PDA nanoparticles, thus promoting its stability. What's more, gas therapy represented by Nitric Oxide (NO) has the advantages of killing bacteria, promoting angiogenesis, and accelerating wound healing. N,N'-diphenylbutyl-N,N'-dinitroso-1, 4-phenylenediamine (BNN6) is one of the Bis-N-nitroso compounds which was commonly used NO donors with good thermal stability. Therefore, the combination of the photothermal effect of PDA, antibacterial capacity of NO, and biocompatible hydrogel of gelatin methacrylamide (GelMA) may promote the healing of bacterial infection wounds. **METHODS:** BNN6-functionalized PDA encapsulated into GelMA (BNN6/PDA/GelMA) hydrogel was developed. Systemic in vitro experiments were conducted to evaluate the physicochemical, photothermal, and anti-bacterial properties under the irradiation of near-infrared (NIR) light. The cellular experiments were carried out to estimate the biocompatibility of hydrogel. Finally, the hydrogels were placed in a rat wound infection model to evaluate their antibacterial and wound-healing abilities in vivo.

RESULTS: The fabricated hydrogel exhibited porous structure and good mechanical properties. When irradiated by 808nm NIR, the BNN6/PDA/GelMA hydrogel demonstrated stably increased temperature within 5 minutes and sustainably released NO from BNN6. Antibacterial tests showed that the BNN6/PDA/GelMA hydrogel possessed broad-spectrum antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* both in vitro and in vivo. Cellular experiments showed that BNN6/PDA/GelMA hydrogel has good biocompatibility. Finally, animal experiments showed that BNN6/PDA/GelMA hydrogel has good antibacterial and accelerated wound healing effects in response to NIR in the treatment of infected wounds.

DISCUSSION & CONCLUSIONS: Under the irradiation of NIR (808 nm), the high temperature induced by PDA can kill bacteria. What's more, this high temperature can promote the release of NO from BNN6, thereby synergistically enhancing the anti-bactericidal effect. At the same time, NO can also accelerate wound healing by promoting collagen deposition. Our work provides new ideas for constructing a BNN6-functionalized PDA photothermal system combined with NO for antibacterial therapy and wound healing.

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Keywords: Wound healing, Composite materials

OP-181

Integrative tissue and plasma proteomics revealed liquid biopsy-accessible indicative biomarkers for impaired bone fracture healing in type 2 diabetes mellitus

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INTRODUCTION: Type 2 diabetes mellitus (T2DM) can lead to compromised fracture healing, but to highly patient-specific extent. Yet, no indicative biomarker profile, to identify diabetes patients at risk, has been described. Moreover, the molecular mechanisms that lead to impaired fracture healing at diabetic conditions are not completely understood. In the interdisciplinary SyMBoD project, that combines animal models and clinical sampling with Omics techniques and systems medicine, we aim to collect, analyze and provide molecular profiles in a digital platform to identify high-risk patients and develop image-guided personalized and functionalized polycaprolactone (PCL)-based scaffolds, that address the molecular needs at diabetic conditions.

METHODS: The PCL-guided regeneration was investigated in a critical-size femur defect model in diabetic rats. A longitudinal sampling of blood plasma was performed over 42 days post-surgery. Of the same animals, regenerated tissue was explanted at 21 days or 42 days post-surgery. Multiplexed proteomics screening analyses were carried out of all collected samples on a QExactive HF mass spectrometer and processed by MaxQuant. Proteins altered during bone regeneration at diabetic conditions were identified by (i) pairwise correlations of disease conditions and time points, (ii) time-aware network-constraint biclustering and (iii) *de novo* network enrichment.

RESULTS: Deep proteomics screening of bone tissue enabled the profiling of more than 4,000 protein and thus, provided novel insights in molecular dysregulations at diabetic conditions. By including non-injured contralateral controls, we are able to discriminate baseline effects of diabetic disease from those related to PCL scaffold-guided bone regeneration. In parallel, 667 proteins were monitored in blood plasma over the regeneration period with 103 proteins providing altered longitudinal profiles. Direct comparisons revealed 20 proteins to be affected in both bone tissue and plasma including proteins previously related to bone regeneration and diabetes. For 14 proteins, abundance profiles in plasma reflect those in tissue. Further, *de novo* network enrichment analyses enabled the functional linkage of profile-matched proteins in plasma and tissue.

DISCUSSION & CONCLUSIONS: To the best of our knowledge, we report the first list of proteins that include altered proteins that are accessible from liquid biopsies and are directly or functionally linked to local effects over the course of bone regeneration. Thus, those proteins may serve as indicative biomarkers to predict bone regeneration capacities. Further, deep proteomics profiling revealed dysregulated functions and pathways at diabetic conditions, that may serve as targets for functionalization of PCL scaffold that address the needs of diabetic patients in impaired bone healing.

Keywords: Bone and bone disorders (osteoporosis etc), Omics / bioinformatics and systems biology

OP-182

Hierarchically Patterned Silk Fibroin Porous Scaffold Transpires as a Potential Deep Cutaneous Healing Implant by Providing Cues to Fibroblast Sensors and Offering Controlled and Replenishable Ampicillin Delivery

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INTRODUCTION: Targeting the aspects associated with delay in cutaneous wound healing our group has already developed mesenchymal stem cell behavior modulating silk fibroin scaffolds[1]. However, perceiving the unmet need for a holistic approach we decided to impart biophysical cues by biomimetically patterning silk fibroin porous scaffold and embed ampicillin within the substrate with a drug replenishable establishment that would offer an all-inclusive aid to healing of deep cutaneous cuts.

METHODS: A combination of soft lithography and free-drying was applied to develop flat (FASF), lotus leaf patterned (LPASF), and rose petal patterned (RPASF) silk fibroin scaffolds with a unique intravenous infusion needle attached ampicillin loading and replenishing arrangement. All the scaffolds were tested for surface properties using SEM, AFM, and contact angle goniometry; chemical properties using FTIR, XRD, XPS, and EDS; ampicillin release kinetics; antibacterial property against *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) using agar disc diffusion method; fibroblast-matrix interaction and functional modeling; cellular infiltration and molecular dynamics of collagen I, vinculin, and vimentin expressions using immunofluorescence studies.

RESULTS: RPASF with an average surface roughness of $1.60 \pm 0.02 \mu\text{m}$ and a contact angle of $159.5 \pm 0.71^\circ$ showed least degradation and a controlled release of ampicillin up to 32 days with successful drug replenishment. Remarkable antibacterial activity was shown by RPASF against both MRSA and *E.coli*. The integrity of cell membranes was not disturbed by RPASF surface features and this substrate best supported rapid cellular infiltration and integration. RPASF supported complete fibroblast sheet formation with the highest cell viability and proliferation ($93.69 \pm 2.91\%$) and also underpinned expressions of vinculin, collagen I, and vimentin after 5 days of in vitro culture.

DISCUSSION & CONCLUSIONS: The superhydrophobic surface of RPASF provided biophysical cues to the fibroblasts to form a cellular monolayer analogous to granulation tissue formation in the proliferative stage of wound healing. We modeled functionally distinct cell-matrix cross-talk due to biophysical cue-steered signaling which concurrently governed the controlled release of ampicillin. Notably, we were successful in delivering a hydrophilic drug by hydrophobically modifying a polymer surface without any chemical intervention. Besides this, rapid fibroblast infiltration within the scaffold followed by high expressions of vinculin, collagen I, and vimentin proved that the RPASF could be used as a standalone deep cutaneous healing supporting holistic tool.

ACKNOWLEDGEMENTS: Ministry of Human Resource Development, Government of India.

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Keywords: Drug delivery, Biofabrication

OP-183

Investigating the antimicrobial effects of metal-doped bioactive glass fibres on chronic wound biofilms

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INTRODUCTION: Chronic wounds (principally pressure sores, venous leg ulcers and diabetic foot ulcers) are a drain on global health services and remain a major area of unmet clinical need[1]. Bioactive glass fibres, which have a “cotton-wool” appearance, offer a promising treatment for chronic wounds[2]. Fibres are created by electrospinning inorganic sol-gel solutions made up of SiO₂ and CaO. Fibres form a 3D structure that mimics the fibrin clot and is both mesoporous and macroporous with large inter-fibre spaces. This morphology makes the fibres ideal for packing into wounds, even with complex geometries. Moreover, fibres have a high surface area to volume ratio which makes them highly reactive. When fibres are placed in the fluid, they undergo multiple chemical reactions such as leeching, dissolution and precipitation. Si⁴⁺ and Ca²⁺ ions released during dissolution can promote wound healing[3]. Here we investigate the anti-biofilm properties of fibres doped with Ag⁺ and Cu²⁺, which are known to have antimicrobial activity[4,5].

METHODS: *Pseudomonas aeruginosa* biofilms were treated with undoped fibres (only containing silica and calcium oxide) or fibres doped with either Ag⁺ or Cu²⁺ ions. Biofilm viability assays were performed, and SEM and confocal images were taken to illustrate biofilm structure before and after treatment. Treatments involving a single application of fibres were compared to treatments involving two sequential applications of fibres, where copper-doped fibres were applied followed by silver-doped fibres or 2 applications of silver-doped fibres were applied.

RESULTS: Treatment with silver-doped fibres reduced biofilm viability much further than copper-doped fibres and undoped fibres. Similar reductions in viability were observed for biofilms treated with undoped fibres and copper-doped fibres. However, there was a larger reduction in viability in biofilms treated with copper-doped fibres followed by silver-doped fibres rather than 2 sequential treatments of silver-doped fibres.

DISCUSSION & CONCLUSIONS: As a single treatment, copper appears to have a minimal antimicrobial effect as copper-doped fibres had similar reductions in viability to undoped fibres. However, there are strong synergistic interactions between copper-doped and silver-doped fibres, resulting in a lower biofilm viability compared to 2 applications of silver-doped fibres.

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Keywords: Drug delivery, Infection

OP-184

Quaternized chitosan/graphene oxide/collagen scaffolds with efficient hemostatic and antibacterial properties for infected wound healing

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INTRODUCTION: Infectious wound has caused substantial economic losses and considerable threats to global health. The traditional treatment for bacterial infections lay on antibiotics. However, the overuse of antibiotics has led to multidrug-resistant (MDR) bacteria. Therefore, developing alternative therapeutics to eliminate bacterial infections without inducing MDR has become an emergent goal. Collagen (Col), the main component of skin, has been widely used to develop wound dressing due to its good biocompatibility and hemostatic performance. While the insufficient antibacterial and poor mechanical properties hindered its further application. Quaternized chitosan (QCS) is a cationic polymer with good antibacterial properties and has been widely used to construct antibacterial wound dressings. Graphene oxide (GO) is an inorganic nanomaterial with superior mechanical properties and abundant functional groups, which ensure that GO-based composite materials with good mechanical properties. Therefore, we develop a QCS-GO-Col scaffold that may have the potential to possess good mechanical and antibacterial properties, which may accelerate the repair of infected wounds.

METHODS: We used a one-step method to prepare a QCS-GO-Col sponge porous scaffold by cross-linking and lyophilization. Systemic in vitro and in vivo experiments were conducted to evaluate the physiochemical, antibacterial, and hemostatic properties and to determine the effect of the scaffold on cell viability, cell proliferation, and osteogenic differentiation and its ability to repair the infected wounds.

RESULTS: We first prepared a QCS-GO-Col scaffold with a porous and interconnected structure under the observation of scanning electron microscope. The FT-IR, XRD, and Raman spectrum experiments suggested the successful cross-linking and preparation of the QCS-GO-Col scaffold. The fabricated scaffold showed good mechanical, antibacterial (*Staphylococcus* and *E. coli*), and hemostatic properties in vitro. Furthermore, no significant biotoxicity was observed when cultured with fibroblasts. Finally, the designed QCS-GO-Col scaffold was demonstrated to have superior antibacterial efficacy in vivo and accelerate the repair of infected wounds. Histological staining suggested that the QCS-GO-Col scaffold promoted wound repair by inhibiting inflammation and promoting angiogenesis.

DISCUSSION & CONCLUSIONS: In this study, we successfully prepared a QCS-GO-Col scaffold, which has good mechanical, antibacterial, and hemostatic properties, and accelerated the repair of infection wounds by destroying bacterial structure, inhibiting inflammation, and promoting angiogenesis.

Keywords: Wound healing, Biomaterials

OP-185

Towards a bioprinted spinal cord model for monitoring cell responses to particles and ions released from spinal implants

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INTRODUCTION: Spinal implants may release ions and particles, and there is a need to understand the biological responses triggered by such debris - in particular the immune response is under-researched. In the spinal cord the innate immune system is represented by microglia. Astrocytes are also well-established contributors to inflammation. A recent study established that the viability of glial cells dispersed in a manually dispensed 3D collagen gel was reduced by cobalt chrome (CoCr) wear particles¹, but otherwise there is a lack of *in vitro* model systems for studying such debris-triggered responses. The aim of this study is to create a bioprinted spinal cord model closely mimicking the *in vivo* situation that will enable direct monitoring of cell responses to wear debris by time-lapse fluorescence microscopy.

METHODS: Laminin was chosen as the basis for bioinks since laminins are important extracellular matrix (ECM) glycoproteins found in the basement membrane and provide adhesion sites for pro-inflammatory microglia². Our in-house developed open source bioprinter³ was used to extrude a laminin-based hydrogel (GelXA Laminink 411, CellInk) containing suspensions of microglia (C8-B4 cell line) and/or astrocytes (C8-D1A cell line). These glial-laden hydrogel constructs were exposed to model particles from materials used in spinal implants. Cell viability in the constructs was monitored by time-lapse imaging of fluorescent reporters and inflammatory activation assessed by immunocytochemistry.

RESULTS: We have established that bioprinting can be used to create advanced 3D tissue models using glial cells, and that cell viability in response to different treatments can be monitored using time-lapse imaging of the caspase-3/7 apoptosis reporter and propidium iodide labelling for cell death.

DISCUSSION & CONCLUSIONS: By using a laminin-based hydrogel we incorporated a key component which is known to influence microglial inflammatory responses that has not been including in similar models before. An automated bioprinting strategy was in this study proposed to create a more relevant and reproducible 3D spinal cord model compatible with high throughput imaging, which will permit analysis of a wide range of debris particle concentrations and combinations.

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Keywords: Hydrogels and injectable systems, Biomaterials

OP-186

3D printing microfibre scaffoldings for cell alignment and in situ sensing

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INTRODUCTION: Micro-fibres, with diameters similar to the size of biological cells, could trigger cell-fibre interactions. Previous studies have shown that micro-fibres made of biocompatible materials could facilitate cell attachment and alignment, so that the fibre network structures could be made into in vitro cell culture scaffoldings [1]. However, the microfibres used in previously reported works are made of electrically-passive materials, lacking the ability for in situ sensing and stimulation. Herein, we demonstrate a 3D printing approach to fabricate biocompatible and conducting fibre network that could simultaneously trigger cell interactions and record the cell attachment and proliferation process.

METHODS: Conducting PEDOT:PSS [poly(3,4-ethylenedioxythiophene) polystyrene sulfonate] fibres with average diameter of 2 μm are printed using Inflight Fibre Printing technique [2]. Because the cell membranes are insulating, the cell attachment on the fibres would result the impedance increase that could be detected through impedimetric spectroscopy.

RESULTS: First we show that the conducting PEDOT:PSS fibre arrays are highly biocompatible and could trigger cell attachment. Thanks to the conductivity of the fibres, the cell attachment process in the first 5 hours after cell seeding could be shown by the impedance change of the fibres.

DISCUSSION & CONCLUSIONS: This work could benefit research for continuous cell monitoring when imaging is not accessible, and could potentially pave way for future study of 3D cell culture and bioelectronic sensors and models.

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Keywords: Interfaces - engineered, Additive manufacturing

OP-187

Construction of a Biodegradable Bioartificial Pancreas to Treat Type 1 Diabetes

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INTRODUCTION: Type 1 Diabetes is an autoimmune condition which, through beta and immune cell dysfunction causes the progressive removal of the body's ability to produce insulin. As a result, researchers have suggested the use of a bioartificial pancreas, combining the insulin secreting function of living beta cells with the immune-protective barrier within a stress-shielding construct. Here we demonstrate the construction of a bioartificial pancreas using a novel biodegradable medium chain length Polyhydroxyalkanoate

METHODS: The mcl-PHA polymer was produced over the course of a 24 hour fermentation using butyric acid as the carbon source. The resultant polymer was washed, homogenized and purified using Soxhlet extraction technique. The polymer was subjected to FTIR, DSC, GTA, GPC and GC-MS. BRIN-BD11 beta cell lines were cultured and encapsulated in a 2% sodium alginate solution. Both materials were printed using multimaterial fused deposition melting. Constructs were tested for viability over 4 weeks as well as stained for insulin secretion and ELISA-measured insulin release in glucose-supplemented cultures. Structures were also weighed following 30 days in media to determine degradation rate.

RESULTS: The confirmatory techniques above confirmed the polymer as an mcl-PHA containing Poly (3, hydroxyoctanoate) monomers. Constructs were successfully printed and the encapsulated BRIN-BD11 displayed preserved viability over 4 weeks in cell culture conditions, as well as demonstrating islet-like morphology. Cells stained positive for insulin and constructs displayed glucose-stimulated insulin secretion at 1,2,3 and 4 weeks. Constructs displayed surface degradation during the testing period, with 2% weight loss detected at 30 days

DISCUSSION & CONCLUSIONS: In this work we demonstrate the ability of a biodegradable bioartificial pancreas to produce physiological relevant quantities of insulin in response to glucose concentration. We also demonstrated the long term survival of a beta cell line within a multimaterial pancreas construct, as well as the biodegradability of the final design. Future work aims to functionalize the stress shielding portion of the bioartificial pancreas to include moieties capable of oxygen production and revascularisation.

ACKNOWLEDGEMENTS: JH thanks Professors Victor Gault and Nigel Irwin at Ulster University for BRIN-BD11 cells, as well as University of Sheffield and EPSRC for JH's scholarship.

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Keywords: 3D printing and bioprinting, Immunity / immunomodulation / macrophage

OP-188

Nerve regeneration by Bio 3D nerve conduit using a bio-3D printer for peripheral nerve injury

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INTRODUCTION: Autologous nerve grafts are the gold standard in peripheral nerve surgery. However, they involve donor site morbidities such as loss of sensation and painful neuromas due to the sacrifice of healthy nerves. Artificial nerve conduits have been developed around the world that consist solely of a scaffold, without cells, regenerative factors, or blood circulation. In artificial nerve conduits, efficient nerve regeneration has not yet been achieved. Therefore, we focused on bio-3D printer technology, which can construct three-dimensional tissues only with cells, and developed a three-dimensional nerve conduit (Bio 3D nerve conduit) with fibroblasts. The purpose of this study is to confirm nerve regeneration in the rat and canine peripheral nerve gap models and to start an investigator-initiated clinical trial for the treatment of a traumatic peripheral nerve defect in the hand.

METHODS: In the rat nerve defect model, a 5-mm nerve defect was created at the right mid-thigh level of the sciatic nerve in immunodeficient rats, and the nerve gap was bridged using 8-mm Bio 3D nerve conduits fabricated from human fibroblasts. In the canine nerve defect model, dermal fibroblasts were isolated and cultured from canine inguinal-region skin to fabricate a Bio 3D nerve conduit with a 5-mm diameter for 8 weeks. A 5-mm nerve defect was created at the ulnar nerve in the forelimb, and the nerve gap was bridged using 8-mm Bio 3D nerve conduits.

RESULTS: In the rat model, the nerve gaps were bridged successfully in all rats and significantly better nerve regenerations than the control group were confirmed kinematically, electrophysiologically, histologically, and morphologically.

In the canine model, the ulnar nerves were successfully bridged using Bio 3D nerve conduits in all canines. The pinprick test indicated functional nerve recovery and the compound muscle action potentials of the hypothenar muscles were detected. Histological evaluation showed numerous well-myelinated axons in the Bio 3D nerve conduit group. No adverse events were observed.

DISCUSSION & CONCLUSIONS: We obtained good nerve regeneration results from the rat model and non-clinical proof of concept using the canine model. Based on these results, an investigator-initiated clinical trial was initiated to examine the safety and efficacy of Bio 3D nerve conduit transplantation for peripheral nerve injury. Bio 3D nerve conduit is prepared from the groin skin for 8 weeks, and then transplanted to a traumatic peripheral nerve defect in the hand.

Keywords: 3D printing and bioprinting, Trauma / surgery and rehabilitation

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Autologous transplantation of bioprinted auricular cartilage in a rabbit model

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INTRODUCTION: The current gold standard of auricular reconstruction entails the carving and assembly of an auricular construct from autologous costal cartilage. The procedure is highly tedious and skill-dependent, requires multiple surgical interventions, and may lead to donor-site morbidity. Tissue engineering (TE) provides an alternative approach for producing a pinna that can be tailored to the patient individually. A novel bioink composed of hyaluronan transglutaminase (HA-TG) employs calcium-triggered enzymatic crosslinking and can support maturation of TE-cartilage with near-native mechanical properties using autologous human auricular chondrocytes. For translation of this technology into the clinic, immunocompetent animal models are urgently required. Thus, we investigated this bioink in an autologous rabbit model, which provides a model close to clinical auricular reconstruction.

METHODS: Autologous rabbit auricular chondrocytes were harvested from a biopsy of approximately 2 x 2 cm and expanded in culture. The chondrocytes were then embedded in a bioink made of HA-TG mixed with alginate (HA-TG-Alg) and cylindrical constructs with a diameter of 1 cm were bioprinted at the Laboratory of TE and Biofabrication (TEB) at ETH Zurich. The autologous constructs were allowed to mature in vitro for 9 weeks before being transplanted between the vascularized panniculus carnosus muscle and fascia at the dorsum of the donor animal. Cell-free cylinders and autologous ear cartilage were transplanted as controls. All transplants were harvested after 13 weeks. Biocompatibility was evaluated by histological analysis, and cartilage-relevant proteins were detected by immunohistochemistry.

RESULTS: Surgical preparation of the panniculus carnosus is technically feasible in a rabbit model. Macroscopically, the shape of the transplants was retained; however, the histological analysis revealed fibrous encapsulation and varying degrees of peripheral degradation with inflammatory cell infiltration in all HA-TG-Alg implants. The analysis of biocompatibility showed moderate to strong irritation in response to the TE samples. Autologous transplants did not reveal inflammatory cell infiltration in a remarkable manner. Characteristic collagen II and safranin O staining were present in autologous but not in the HA-TG-Alg transplants.

DISCUSSION & CONCLUSIONS: The panniculus carnosus is a suitable model for a temporalis fascia flap, which is currently used in clinical auricular reconstruction with rib cartilage. Despite previously demonstrated successful maturation in the immunocompromised xenograft model, strong to moderate immune response to the transplanted TE constructs accompanied by the disappearance of typical cartilage histology was observed irrespective of the cell content. Further modifications of the bioprinted constructs are thus required.

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Keywords: 3D printing and bioprinting, Cartilage / joint and arthritic conditions

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Tomographic volumetric bioprinting of 3D pancreatic cancer models

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INTRODUCTION: The pancreatic cancer is the one of the leading causes of cancer-related death worldwide with a five-year survival rate of below 9%¹. Although recent studies have shown the possibility of modeling the tumor microenvironment (TME) *in vitro*², the tumor-stroma crosstalk remains extremely challenging to be reproduced and monitored in functionally effective models. Moreover, most of the works in literature lack to resemble the native compartmentalized TME architecture that is widely recognized to affect cancer cells response to therapeutics². The volumetric tomographic printing represents an emerging light-based technology capable of fast fabricating cellularized 3D constructs with high-resolution and complex geometries³. Therefore, we adopted this innovative technique to develop a biomimetic 3D *in vitro* model of the human pancreatic gland.

METHODS: Human fibroblasts (HFF1) were embedded in a Gelatin-Methacryloyl (GelMA) hydrogel, prepared tailoring the polymer and the photoinitiator concentrations, and the complex glandular structure has been obtained through photopolymerization by volumetric additive manufacturing. The HFF1 viability inside the printed gels was monitored by CellTiter-Blue™ and LIVE/DEAD™ assays at different time points. Human pancreatic ductal epithelial cells (HPDE) stably expressing activated KRAS (HPDE-KRAS), were then seeded within the cavity of the bioprinted constructs to develop a fully human pancreatic cancer model. The stromal and pancreatic cancer cells crosstalk was evaluated by quantifying the expression of markers responsible for fibroblasts activation (i.e. alpha smooth muscle actin, α -SMA).

RESULTS: The viability tests showed that fibroblasts remain viable up to 14 days within the bioprinted GelMA constructs, confirming the cell-friendly light based volumetric bioprinting procedure. In addition, the results demonstrated that HPDE-KRAS cells were able to entirely cover the inner walls of the construct by growing as an epithelial monolayer. Finally, the tests performed also proved that human fibroblasts embedded in GelMA hydrogel show an increment in α -SMA expression when co-cultured with HPDE-KRAS compared to HFF1 alone and to fibroblasts seeded with normal HPDE cells.

DISCUSSION & CONCLUSIONS: This 3D *in vitro* model allows to recapitulate the complex microanatomy of the exocrine pancreas and study the mechanisms that take place during the early stages of the pancreatic cancer. Therefore, it represents a powerful tool for the development of new diagnostic and therapeutical approaches.

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Keywords: Biofabrication, In vitro microenvironments

OP-191

Drop-on-demand micropatterning of novel amphiphilic peptide I3K on regenerated silk fibroin substrates to guide and promote adhesion and proliferation of neuronal cells

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INTRODUCTION: Silk is a natural biomaterial that can be obtained from silkworms such as *Bombyx mori*. It is composed of two proteins called: sericin and fibroin¹. After removing the silk sericin, the remaining silk fibroin is dissolved and dialyzed generating an aqueous solution known as regenerated silk fibroin (RSF). Although RSF can be used as a biomaterial in tissue engineering because of its high biocompatibility and biodegradability, it lacks the needed biological activity to support the adhesion and proliferation of cells^{2 3}. In order to improve cell attachment and proliferation the novel amphiphilic peptide Ac-IHK-NH₂ (I3K) was patterned onto the silk surfaces using a drop-on-demand (DOD) inkjet printer.

METHODS: *B. mori* cocoons were degummed, rinsed and dried. Then the dried fibres were dissolved in Ajisawa's reagent. The synthetic peptide I3K was dissolved in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 6.0). RSF was spun coated on cover slips; then, used as substrate for subsequent printing with a custom-designed inkjet printer equipped with a 40 µm nozzle diameter jetting device. Finally, the PC12 cell line was used to evaluate cell attachment, proliferation and differentiation.

RESULTS: RSF solutions obtained after degumming cocoons during three different times (i.e. 30, 60 and 90 min) showed complete removal of sericin but different behaviour in cell attachment and biocompatibility after dissolution. For subsequent experiments, an RSF solution with low cell attachment was chosen. The combination of I3K and RSF via Van der Waals forces showed an improvement on cell adhesion and proliferation. The effect of self-assembly time of I3K and its concentration was also studied. Finally, the peptide was micropatterned on RSF substrates to guide cell attachment, showing that cells grow only on top of the I3K patterns.

DISCUSSION & CONCLUSIONS: This study demonstrated that even though silk fibroin in its regenerated form, RSF, is biocompatible and exhibits no detectable cytotoxicity on PC12 cells, it lacks the biological activity to readily support cell adhesion. Moreover, the use of DOD inkjet printing allows for the precise deposition of I3K to form high-resolution micropatterns. These can be used to guide and promote cell adhesion to specific locations when combined with an antifouling surface such as RSF.

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Keywords: 3D printing and bioprinting, Nervous system (brain-central-peripheral / disorders)

OP-192

High-throughput quantification of single cell matrix deposition using Extracellular Protein Identification Cytometry (EPIC)

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INTRODUCTION: Extracellular matrix (ECM) analysis is one of the main methods to investigate cellular behaviour, including stem cell differentiation, pathophysiology, and drug responsiveness. Conventional ECM analysis such as western blot, mass spectroscopy or confocal fluorescence imaging of immunostaining have a destructive nature and/or are incompatible with high-throughput single-cell readouts. Consequently, heterogeneity within large cell populations is masked. Here, we present Extracellular Protein Identification Cytometry (EPIC) as a novel method that enables the quantification of specific pericellular matrix proteins of single cells within a 3D microenvironment in a high-throughput and non-destructive manner.

METHODS: Single cell encapsulation of human chondrocytes was performed with two distinct microfluidic chip designs, one for droplet generation and another for delayed gelation [1]. Cells were microencapsulated in 5% Dextran-Tyramine, enzymatically crosslinked using horseradish peroxidase and H₂O₂. To obtain the highest purity of single cell microgels, one in ten microgels contained a cell, as dictated by the Poisson distribution [2]. Encapsulated cells were cultured in chondrogenic medium for three weeks. For indirect immunostaining, microgels were fixed, permeated and subsequently blocked. Imaging was performed with a confocal microscope and a FACS Aria II was used for flow cytometry analysis.

RESULTS: Encapsulated single cells could be cultured for three weeks with minimal cell escape [1], which allowed for ECM deposition. Subsequent live-dead staining confirmed high cell viability. As a proof of principle, chondrogenic differentiation was assessed via immunostaining of cell-laden microgels for COL I and COL VI. Confocal microscopy verified that the labelled matrix was located extracellularly. Quantitative analysis was performed with flow cytometry at a rate of up to 1000 events per second. DAPI staining allowed for distinguishing between empty and cell-laden gels, and an isotype control was taken along to correct for non-specific binding. Gating for the single cell microgels revealed that about 80% deposited COL I and about 23% deposited COL VI detectably. Moreover, levels of matrix deposition varied between individual cells revealing heterogeneity in collagen deposition.

DISCUSSION & CONCLUSIONS: EPIC enabled the detection of immunolabelled deposited ECM at single cell resolution in high-throughput. Moreover, we demonstrated that EPIC has the potential to quantitate matrix deposition of individual cells in 3D hydrogels, which allows for subpopulation analysis within engineered microenvironments.

ACKNOWLEDGEMENTS: Financial support was received from the European Research Council (ERC, Starting Grant, #759425) and Dutch Research Council (NWO, Vidi Grant, #17522).

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Keywords: Microenvironment and niche engineering, Enabling technologies

OP-193

Time-of-day secretome profiling reveals rhythmic ECM secretion and altered circadian ECM remodelling during skeletal muscle ageing

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INTRODUCTION: Skeletal muscle growth and repair are highly regulated processes controlled by interactions between muscle cells and their surrounding niche, including secreted growth factors and extracellular matrix (ECM). The importance of circadian rhythms, a ~24h time-keeping molecular mechanism, for skeletal muscle is evident from altered skeletal muscle phenotypes in mouse models of genetic clock disruption and in human studies. Moreover, muscle-specific genetic clock ablation leads to systemic effects on the entire musculoskeletal system, including bone, tendon and cartilage. Recent work has implicated an important secretory function for skeletal muscle through secreting molecules that affect local muscle ECM in an auto/paracrine manner as well as having endocrine effects on other tissues. However, the role of circadian rhythms in their timely regulation, or their feedback effects on myogenesis and muscle ageing is not known.

METHODS: Here we used a quantitative proteomics platform to investigate the secreted factors regulated by time-of-day cues in differentiating murine skeletal muscle progenitor cells. We performed loss- and gain-of-function experiments alongside real-time bioluminescence imaging of muscle cells containing clock gene luciferase reporters to assess the effects of muscle secreted factors on muscle cell clocks and their differentiation process. Circadian gene/protein expression analyses was used to determine their rhythmic expression in muscle cells and tissues from young and old animals alongside their activation following in vivo time-of-day growth factor and neuromuscular stimulation.

RESULTS: Our data revealed that the differentiating muscle cell secretome is regulated in a time-of-day manner, including structural and remodelling ECM components as well as signalling factors. This is accompanied by increased amplitude and rhythmicity of the muscle cell intrinsic clock, in part, reinforced by the feedback from the secreted signalling factors such as pro-anabolic muscle regulators. Moreover, many validated secreted proteins, are regulated through the transcriptional molecular feedback loops and show circadian expression both in muscle cells in vitro and muscle tissues in vivo. In particular, we show time-of-day ECM gene regulation in response to timed growth factor administration or muscle stimulation in vivo. Finally, we reveal dampened amplitude of the molecular clock and altered time-of-day transcriptional regulation of clock-controlled secreted ECM factors in ageing skeletal muscles.

DISCUSSION & CONCLUSIONS: These data implicate an important function for circadian timing in maintenance of appropriate muscle ECM microenvironment during muscle differentiation and necessitate future development of chrono-based interventions for restoring ECM microenvironment in age-related sarcopenia and human muscle-related pathologies.

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Keywords: Stem cell niche, Differentiation

OP-194

A 3D in vitro bone model with primary human osteocytes and osteoclasts, simultaneously differentiated in a triple culture with osteoblasts

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INTRODUCTION: With a worldwide aging population, research in bone metabolism and novel therapies for damaged and diseased bone is essential. We recently developed an in vitro human bone model, comprising the three main bone cell species – osteoblasts, osteoclasts and osteocytes, isolated from primary human tissue (1). Due to divergent media requirements, the different cell species were differentiated prior to the assembly of bone triple cultures. This model was suitable for the investigation of factors with proven impact on bone metabolism like Sr^{2+} , hypoxic conditions (2) or degradation products of Mg-based implants (3). However, the established model was not suitable to analyse the impact of different factors on osteoclasts formation as well as osteocyte differentiation.

METHODS: Osteoblasts were isolated from human femoral heads. Osteocytes were differentiated from osteoblasts as described before (1), osteoclasts were differentiated from peripheral blood mononuclear cells (PBMC). All three cell types were separated from each other to allow exchange of soluble signaling molecules and separate analysis of the cell specific markers. Triple cultures were conducted for 14 days. Microscopic investigations, gene expression analysis as well as quantification of cell type specific enzyme activities were performed.

RESULTS: Comprehensive tests were performed to find conditions allowing the simultaneous differentiation of osteocytes from osteoblasts and the formation of osteoclasts from PBMC. In the new triple culture model, morphology, gene expression and marker enzyme activities of the three bone cell species like alkaline phosphatase for osteoblasts, tartrate resistant acid phosphatase and cathepsin K for osteoclasts were established and characteristics for all the differentiated bone cell species were demonstrated.

DISCUSSION & CONCLUSIONS: We demonstrated that the external addition of cytokines like macrophage colony-stimulating factor (MCSF) or receptor activator of nuclear factor- κB ligand (RANKL) for the differentiation of multinucleated osteoclasts from PBMC is not necessary in the triple culture and displays a more physiologically relevant culture system. This improved bone model might help to study and understand the complex crosstalk of bone cells and their precursors during remodeling of bone tissue. Moreover, it enables preclinical testing of various parameters like the impact of bioactive factors, biomaterial extracts or drugs on the differentiation and behavior of bone cells, for translation into clinical practice. With this bone model, we are aiming to reduce the amount of in vivo animal studies in bone research.

ACKNOWLEDGEMENTS: DFG (BE5139/3-2)

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Keywords: Bone and bone disorders (osteoporosis etc), Differentiation

OP-195

Harnessing Stem Cell Response to Viscoelasticity

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INTRODUCTION: The cellular microenvironment is a mechanically complex, dynamic niche that exhibits time-dependent responses to deformation [1]. Despite this viscoelastic behaviour, materials utilised in studies of cellular mechanotransduction are generally purely elastic and don't recapitulate the true mechanical environment experienced by cells in vivo. Since tissue mechanics play a key role in development, ageing, cancer progression and disease, elucidating how cells sense and respond to a dynamic mechanical environment is crucial for a thorough understanding of cellular mechanobiology and tissue homeostasis. Recent research has investigated the role of viscoelasticity; however, conflicting results have been reported, highlighting that our knowledge in the field is still limited [2]. Here, we aim to address these shortfalls in knowledge in mechanobiology by developing robust material platforms with fully controllable viscoelastic properties, and by investigating the mechanotransductive mechanisms of cell response.

METHODS: We developed 2D polyacrylamide hydrogels with distinct viscoelastic properties in a physiologically relevant range (Young's modulus of 1 - 7 kPa; loss tangent of 0.05 - 0.3) and functionalized them with different protein-derived adhesive ligands (RGD from fibronectin and IKVAV from laminin) to investigate the integrin-dependent response of human mesenchymal stem cells (hMSCs) to material viscoelasticity. We utilized a variety of biophysical and biomolecular techniques, including traction force microscopy, single cell force spectroscopy, atomic force microscopy, immunofluorescence microscopy and qPCR to characterize hMSC behaviour.

RESULTS: We found that hMSC mechanoreponse to viscoelastic interactions depended on the hydrogel stiffness. Specifically, increasing the viscosity of soft hydrogels (<1kPa) enhanced cellular adhesion, traction and spreading, while the opposite trend was observed on stiffer hydrogels (>3kPa), independently of the ligand. Furthermore, we observed mechanoactivation of Yes-associated protein (YAP) in spread cells on rigid elastic hydrogels, particularly in the presence of RGD, but not in cells spread on soft viscoelastic hydrogels. This indicates a mechanical disconnect between cellular traction and nuclear mechanotransduction on soft viscoelastic hydrogels. Ultimately, the differential activation of mechanosensitive pathways altered lineage commitment.

DISCUSSION & CONCLUSIONS: We engineered a hydrogel platform with controlled viscoelastic properties and ligand presentation, and demonstrated the dependence of hMSC mechanoreponse to viscoelasticity on stiffness and ligand type. Our results will allow us to establish a paradigm of stem cell response to viscoelasticity, presenting an avenue for the design of next generation viscoelastic biomaterials for mechanobiology and regenerative medicine.

ACKNOWLEDGEMENTS: MRC funding (MR/S005412/1) is acknowledged.

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Keywords: In vitro microenvironments, Multipotent (mesenchymal) stem cells

OP-196

Decellularised cell-derived matrix enables to study the extracellular matrix 3D remodelling in liver fibrosis and to evaluate therapy response

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INTRODUCTION: Every year, 1 million deaths are associated with liver fibrosis, which is characterised by aberrant accumulation of extracellular matrix (ECM) proteins by activated hepatic stellate cells (HSCs), leading to liver failure and/or liver cancer. While many pathways regulating fibrosis have been elucidated, none of these have been proven targetable with anti-fibrotic drugs. Evidence suggests that several factors (aetiology, profibrotic insults, and fibrotic stage) influence ECM production, composition, and deposition. However, mechanisms behind the distinct ECM biochemical and biophysical remodelling are not fully understood, probably due to the lack of relevant in vitro models. Here, we study ECM deposition and 3D remodelling using an in vitro patient-derived tissue-specific model.

METHODS: Primary human HSCs were isolated from liver tissue with different fibrotic stages using the METAVIR fibrotic score: F0 (no fibrosis) to F4 (cirrhosis). Fibrosis was induced using different combinations of pro-fibrotic stimuli (TGF β , IL-6, free fatty acids) and then treated with a cyclophilin inhibitor, a potential therapeutic anti-fibrotic compound currently in clinical trial. Cell activation was confirmed by gene and protein expression analysis. Metabolic activity of activated cells was determined with Seahorse. ECM deposition upon stimulation was characterised for biochemical composition and fibre alignment by decellularisation of cell-derived matrix (CDM) via immunofluorescence, confocal imaging and proteomics.

RESULTS: Human HSCs were successfully isolated and cultured and showed clear activation upon administration of pro-fibrotic stimuli: increased gene and protein expression of activation markers (α SMA, SOX9, collagen), increased proliferation, and high deposition of 3D ECM in culture. Different combinations of insults generated different levels of cell activation and ECM remodelling in treated HSCs. Interestingly, the fibrotic score of the liver tissue of origin influenced HSCs activation, metabolic activity and matrix production, indicating the suitability of our model to mimic patient- and aetiology-specific fibrotic features. Remarkably, treatment with the cyclophilin inhibitor significantly reduced HSCs activation, ECM deposition and the order of organisation of matrix fibres resulting in less organised bundles, indicating remodelling towards a lower stiffness matrix. Proteomic analysis on CDM revealed qualitative and quantitative differences on CDM compositions between the different HSCs lines (F scores) and the different treatment groups.

DISCUSSION & CONCLUSIONS: Here we showed the potential of our patient-derived in vitro model to investigate ECM remodelling in different fibrotic settings and its resolution upon treatment. The

information on fibrillar quantity and alignment between patients and upon different pro-fibrotic insults provides new crucial information on the molecular mechanisms of ECM deposition in liver fibrosis.

Keywords: Decellularised matrices, Disease models

OP-197

Acellular liver resembled a new liver tissue after transplantation in G-CSF-treated cirrhotic rats

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INTRODUCTION: Acellular Liver Scaffolds (ALS) are a promising tool of tissue engineering able to overcome the organ shortage problem. Great potentials of using ALS in health conditions have been shown, however, the ALS in vivo recellularization properties after transplantation in the cirrhotic model remain unclear. The purpose of this study was to evaluate the potential contribution of ALS to hepatic regeneration and in vivo recellularization after partial hepatectomy in granulocyte colony-stimulating factor (G-CSF) pre-treated cirrhotic rats.

METHODS: To address this issue, donor Wistar rats (n=5) were submitted to a surgical procedure to promote liver procurement (approved by the Animal Care Committee CEUA/CCS-UFRJ 097/20). For decellularization, the livers were transferred to a dish to be perfused through the portal vein using an infusion pump at 3 ml/min with water for 2 hours followed by 1% Triton X-100 for 2 hours and SDS 1% for 18h. After decellularization, livers were washed with distilled water for 1 day and then submitted to DNA quantification and histology analysis (H&E). Cirrhotic recipient rats (n=5) previously received 5% of ethanol in drinking water and intraperitoneal injections of carbon tetrachloride (1 ml/Kg) every other day for 8 weeks. Then, they were treated with G-CSF (100µg/Kg/day) for 5 consecutive days and submitted to median lobe hepatectomy (10%) and subsequently to partial ALS orthotopic transplantation. Recipients rat serum biochemical analyzes (albumin (ALB) and alanine aminotransferase (ALT)) were measured before and 30 days post-transplantation. H&E, Gomori's trichrome, Periodic acid–Schiff (PAS), immunohistochemistry (Ki67, CK7), TUNEL, and immunofluorescence (CK19, F4/80) analyzes were performed to evaluate ALS 30 days post-transplantation. The blood flux into ALS was investigated by microcirculation analysis.

RESULTS: Whole liver macroscopy, H&E staining, and DNA quantification analyzes showed that the decellularization process removed the cells and preserved the vascular structure and components of the ALS. Serum biochemical parameters (ALB, ALT) analysis indicated that the presence of the transplanted ALS normalized the ALB and ALT serum biochemical parameters after transplantation. Histological findings revealed an extensive ALS recellularization 30 days post-transplantation. Immune cells, hepatocytes and cholangiocytes were detected. Also, blood vessel-like structures containing blood cells able to allow blood flow were observed along with the transplanted ALS.

DISCUSSION & CONCLUSIONS: Our data suggest that ALS could be used as an alternative option to promote liver regeneration and rapid hepatic mass repositioning after hepatectomies in patients.

Keywords: In vivo and animal models, Biomaterials

OP-198

In vitro modelling of osteoarthritis as platform for testing mirna-based therapeutic polyplexes

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INTRODUCTION: OA is the main chronic form of joint disease. Pro-inflammatory cytokines play a key role in OA pathogenesis by affecting the anabolic activity of chondrocytes in favour of catabolic activity, leading to protease-mediated Articular Cartilage (AC) degradation (1). Herein, we developed an AC in vitro scaffold-free organoids model in Healthy and OA conditions, with the aim of studying the disease evolution at cellular level and testing therapeutic polyplexes.

METHODS: Spheroids of human articular chondrocytes (HC) and immortalised mesenchymal cells differentiated to chondrocytes (Y201-C) were formed in round-bottom 96-well plate and cultured in three conditions: Healthy (DMEM/F12), Early-stage OA (ES-OA) (DMEM/F12+IL-1 β (1ng/mL)+TNF- α (1ng/mL)+IL-6 (10ng/mL)) and Late-stage OA (LS-OA) (DMEM/F12+IL-1 β (5ng/mL)+TNF- α (5ng/mL)+IL-6 (50ng/mL)). Spheroid growth kinetics and metabolic activity were evaluated over 10 days and then spheroids were assembled and cultured for 21 days on gelatin-coated poly(lactic-co-glycolic acid) electrospun membranes (10 spheroids/cm²) (2). Gene expression analysis (SOX9, COL2A1, ACAN, COL1A2, MMP13 and ADAMTS-5), Histology (H&E, Alcian Blue, Picrosirius red) and Immunohistochemistry (Collagen II, Aggrecan and Ki-67) were performed to assess OA markers in all conditions. miRNA-140-5p-based (20 μ M) chitosan polyplexes (250 μ g/mL) effect was evaluated using the model.

RESULTS: HC and Y201-C spheroids showed a diameter decrease over culture in Healthy state compared to OA conditions, where it remained stable. Cell metabolic activity decreased over culture for HC and increased for Y201-Cs in all conditions, but both cells showed a higher metabolic activity in HC-OA condition at day 10. At day 21, cells in OA environment showed significant decreased expression of anabolic markers (COL2A1, SOX9 and ACAN) and an upregulation of catabolic markers (MMP13 and ADAMTS5), as well as a significantly lower production of collagen and glycosaminoglycans, confirmed by immunohistochemistry. Polyplexes uptake was successful and demonstrated their potential as therapeutic nanoparticles, being able to decrease the expression of MMP13 catabolic marker and increase the expression of COL2A1 in OA-induced models.

DISCUSSION & CONCLUSIONS: We have developed a reliable in vitro OA model where the presence of pro-inflammatory cytokines led to: (i) an increased cells metabolism, due to the biosynthesis of OA-related inflammatory and degradative enzymes at day 10; (ii) decreased expression of anabolic genes in favor of catabolic ones after 21 days culture; (iii) lower production of collagen II and aggrecan as effect of the inflammation; (iv) therapeutic effect of miRNA-based polyplexes as OA treatment strategy.

REFERENCES: [1]Kloppenburger et al, Osteoarthritis Cartilage 28;3,2020;

[2]Scalzone et al. Tissue Engineering Part A, 28(1-2):84,2022.

Keywords: Cartilage / joint and arthritic conditions, In vitro microenvironments

OP-199

Nociceptive sensory neurons promote tissue healing via modulating neutrophil and macrophage activity

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INTRODUCTION: The immune system is known to be a key regulator of tissue regeneration¹. Moreover, evidence shows that nociceptive sensory neurons regulates immune activities in the contexts of allergy and infection². However, a crosstalk between sensory neurons and immune cells during tissue healing following acute injury has not been demonstrated. Nociceptors, which constitute a subset of peripheral sensory neurons, carry the Nav1.8 sodium channels and innervate skin and muscle. In injured tissues, nociceptors are activated by noxious signals, such as inflammatory cytokines. Here we aimed at determining whether nociceptive sensory neurons are important for tissue healing.

METHODS: Nociceptors were deleted in mice using a system targeting Nav1.8 expressing cells (denervated mice). We conditionally deleted Ramp-1, a calcitonin gene-related peptide (CGRP) receptor in myeloid cells. Diabetic mice (Leprdb/db) were used as an impaired wound healing model which displays neuropathies. Tissue injury models were full-thickness skin wounds and volumetric muscle loss. We fused CGRP to a high affinity ECM-binding domain to improve its delivery in injured tissues (ECM-binding CGRP). Tissues healing was assessed by histology and immune activities were assessed using flow cytometry, ELISA, and RNA sequencing.

RESULTS: Denervated mice showed delayed skin and muscle healing. Immunohistochemistry revealed that CGRP was expressed by nociceptors in injured tissues. Deletion of Ramp-1 in myeloid cells compromised skin and muscle healing, demonstrating that endogenous CGRP signalling in myeloid cells is critical for tissue healing. By analysing neutrophils and macrophages in injured tissues and using in vitro assays, we showed that CGRP suppresses migration, increases death, elevates efferocytosis and promotes macrophage polarisation toward an anti-inflammatory phenotype. Additionally, delivering ECM-binding CGRP in skin wounds and muscle defects promoted tissue healing by reducing neutrophil and macrophage accumulation and by accelerating macrophage polarisation. Mechanistically, we revealed that CGRP unregulated the ECM protein thrombospondin-1 in both neutrophils and macrophages and directly modulated their activity.

DISCUSSION & CONCLUSIONS: We demonstrated that CGRP-derived from nociceptors promotes tissue healing via modulating neutrophil and macrophages. Moreover, an ECM-binding form of CGRP promoted rapid tissue healing in diabetic mice by mimicking neuro-immune interactions. CGRP may have therapeutic potential to treat poorly healing tissues where dysregulated neuro-immune interactions play a pathologic role.

ACKNOWLEDGEMENTS: The National Health and Medical Research Council and the Viertel Charitable Foundation.

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Keywords: Diabetic healing, Drug delivery

OP-200

Bioengineered spider silk immobilized on a nanofibrous mesh inhibit bacteria adherence and anticipate new prospects for abdominal muscle tissue repair

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INTRODUCTION: The loss of abdominal muscle tissue function together with the high incidence of surgical site infections (SSI) after surgery represents a major clinical problem. Proteins derived from bioengineered spider silk (SS) emerged as a new class of tunable functional polymer materials that can modulate cell response while impairing infection [1]. We aim to explore the biological potential of immobilized bioengineered SS proteins with antimicrobial (AM) peptides (6mer-HNP1) on nanofibrous meshes aiming to repair abdominal muscle tissue biomechanics and prevent infections. **METHODS:** The electrospun polycaprolactone meshes [2] were coated with 2% of the SS proteins (6mer and 6mer-HNP1) [1] and water annealed for 24h to render the coating insoluble in water. The functionalized meshes were characterized in terms of AM properties, and the secondary structure accessed by FTIR and XRD. The biological performance of the biofunctional meshes was studied, namely the metabolic activity, proliferation, and extracellular matrix (ECM) synthesis by culturing C2C12 cells for up to 7 days. The expression of muscle-related markers was evaluated by Western-Blot and RT-PCR. The experiment was made in triplicates, and statistical analysis was performed ($P < 0.05$).

RESULTS: The bioengineered proteins 6mer and 6mer-HNP1 were immobilized at the nanofibers' surface at a maximum concentration of 200 $\mu\text{g mL}^{-1}$ and 250 $\mu\text{g mL}^{-1}$, respectively. The proteins' immobilisation did not affect the SS beta-sheets formation. The adherence and biofilm formation of Methicillin-Resistant Staphylococcus aureus (MRSA) and Escherichia coli (E. coli) was greatly inhibited in the mesh functionalized with 6mer-HNP1, revealing their anti-infective potential. The cytocompatibility of the biofunctional meshes was validated in vitro using a mouse myoblast cell line (C2C12). The overexpression of muscle transcripts confirms the genotype of muscle cell cultured on the biofunctional mesh.

DISCUSSION & CONCLUSIONS: Nanofibrous meshed functionalized with the 6mer-HNP1 inhibited MRSA and E. coli growth, demonstrating their anti-infective potential. The proliferation of muscle cells demonstrate their potential for biomedical applications. These outcomes provide new wisdom using recombinant SS proteins with AM peptides as multifunctional living constructs for abdominal muscle tissue regeneration and repair.

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Keywords: Infection, Polymers - natural / synthetic / responsive

OP-201

Harnessing the power of regulatory T cells to promote immune-mediated bone regeneration in vivo

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INTRODUCTION: The immune system plays a central role in tissue repair and regeneration. A subset of CD4+ T cells, namely regulatory T cells (Treg), have been reported to affect effector immune response creating the appropriate immune environment for successful tissue regeneration. Growing evidence shows their direct effects on progenitor cells' function. Treg have gained interest due to their potential to enhance bone regeneration, which remains under explored. In this study we aimed at elucidating the impact of co-culturing mouse bone marrow derived mesenchymal stromal cells (mMSC) with mouse Treg (mTreg) before in vivo implantation to promote immune-mediated bone regeneration.

METHODS: Primary mMSC and mTreg were isolated and expanded in vitro before being cultured on bi-calcium phosphate scaffold granules (BCP). The following constructs were then randomly implanted subcutaneously in C57 black 6 mice: BCP alone, BCP+mBMSC, BCP+mBMSC+mTreg. After 2 and 10 weeks the samples were harvested and processed for RNA transcriptomics, multiplexed immunoassay and histology. Blood plasma was collected after 2 and 10 weeks. The brachial lymph nodes, draining the implanted constructs were harvested and isolated cells were immunophenotyped by flow cytometry.

RESULTS: Mineralized bone tissue was observed histologically after 10 weeks in the BCP+mMSC+mTreg group only. After 2 weeks, the BCP+mMSC+mTreg group induced a significant decrease in CD4+, CD8+, CD11b+ and CD19+ cells in regional lymph nodes and a reduction in interferon gamma (INF-g) production from CD4+ and CD8+ cells comparable to baseline. However, after 10 weeks an opposite trend was observed, with an increase in all immune cells phenotyped and highest INF-g production from CD8+ and CD19+ cells in lymph nodes draining BCP+mMSC+mTreg group. Multiplexed immunoassay for the protein isolated from the constructs and plasma revealed that pro healing markers IL13 and IL4 were upregulated in BCP+mMSC+mTreg group at 10 weeks. Transcriptomics revealed 236 significantly upregulated genes in BCP+mMSC+mTreg versus only 171 significantly upregulated genes in BCP+mMSC.

DISCUSSION & CONCLUSIONS: Our results demonstrate that a co-culture of Treg with mBMSC before implantation leads to an in vivo local and systemic immunomodulation that in return creates a pro-healing microenvironment. It led to a reduction of INF-g production which otherwise would have inhibited the differentiation of mMSC. Therefore, this resulted in a promoted MSC-mediated ectopic formation of mineralized tissue. Understanding the complex immune response to MSC-mediated bone regeneration will help in the efficient design of immune-mediated cell-based bone regeneration therapies.

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Keywords: Multipotent (mesenchymal) stem cells, Bone and bone disorders (osteoporosis etc)

OP-202

Regulatory T-cell based therapy to promote repair and regeneration in multiple tissues

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INTRODUCTION: The immune system is a master regulator of tissue repair and regeneration. In particular, regulatory T cells (Tregs) have emerged as critical positive modulators of the tissue healing process. Tregs usually accumulate at an injury site about one week post-injury and are known to be critical for the healing of multiple tissues such as skin, muscle, lung, heart and the central nervous system. Studies have also shown that Tregs express molecules with anti-inflammatory and pro-reparative properties. Thus, given their central role in improving tissue healing outcomes, we sought to explore the potential of using Tregs as a therapy to promote tissue healing following injury.

METHODS: To study the therapeutic effect of Tregs, we locally delivered exogenous mouse spleen Tregs via a hydrogel, into injured bone, muscle and skin tissues. We then monitored tissue healing in Treg-treated tissues and controls, across different time points, by histology and microCT techniques. To further determine the mechanisms by which Tregs act within the tissue, we characterised the profile of other immune cells within the injuries, using flow cytometry and bulk RNA sequencing. We also recovered the delivered Tregs, and endogenous macrophages, from Treg-treated tissues at various time points to analyse their phenotypic changes.

RESULTS: Strikingly, Tregs significantly enhanced tissue healing in all tissues tested, and this was consistent across syngeneic, allogeneic and xenogeneic Treg delivery. Mechanistically, we found that the therapeutic effect of Tregs was dependent on macrophages within the injured tissues. Indeed, Tregs were found to induce phenotypic and transcriptional changes in monocytes/macrophages across different time points during tissue healing, as shown by RNA sequencing and immune profiling of macrophages from Treg-treated tissues. This was also accompanied by a reduced accumulation of inflammatory cells and an increase in expression of macrophage markers associated with a pro-healing phenotype. In addition, the delivered exogenous Tregs could be recovered for at least up to 5 days from the injured tissues. Their transcriptional profile showed that they adopted a “tissue Treg” phenotype and expressed genes involved in immunomodulation and tissue repair.

DISCUSSION & CONCLUSIONS: Overall, we found that Tregs play a critical role during tissue repair and regeneration, and exogenous Treg delivery promotes accelerated healing across multiple tissues, largely by modulating macrophages. This demonstrates the potential of Tregs as a novel cell-based strategy to improve regenerative outcomes in multiple tissues.

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Keywords: Cell therapy, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-203

Monocyte-derived macrophages and dermal fibroblasts-derived ECM making small talk

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INTRODUCTION: 3D in vitro skin models allow studying molecular pathways that otherwise are only possible to explore using animal models. The incorporation of immune cells, such as macrophages, has been implemented to create immunocompetent analogues. Yet, the human biology representation in those models is limited, among other aspects, by the minimal extracellular matrix (ECM) mimicry[1]. In order to tackle this limitation, we have established a protocol to extract fibroblasts-own ECM. In this work, we hypothesised that those ECM extracts were not able to promote macrophage activation, which could compromise the physiologic phenotype of the model.

METHODS: Structural (strECM) and soluble (sECM) ECM components were extracted from fibroblasts cultures using an in-house established approach. Blood monocytes were differentiated into macrophages (MDMs) with M-CSF (50ng/mL) and exposed to a range of ECM concentrations (5, 25 and 50 µg/mL - sECM or 250, 500 and 1000 µg/mL - strECM). The response to the ECM was also assessed in the presence of TNFα (50ng/mL and 100ng/mL), to understand its influence on a pro-inflammatory signal. Pro-inflammatory control cells were attained with LPS/IFNγ (10ng/mL, 20ng/mL). MDMs response was monitored after 24h of exposure by RT-PCR (NF-kB, STAT1, PPARG and JMJD3), flow cytometry (CD14, CD86, CD197, CD319, CD206,) and immunofluorescence (CD86, CD206) analyses, and multiplexing of cells' supernatants (IL-6, CXCL10, TNF-α, CCL18, CCL22).

RESULTS: Exposure to the ECM extracts led to downregulation of transcription factors associated with pro-inflammatory pathways (NF-kB, STAT1) and unchanged mRNA expression of anti-inflammatory (PPARG and JMJD3) genes. Moreover, lower/unchanged levels of pro-inflammatory surface markers (CD86, CD197, CD319) were detected. All cytokine's levels were similar to the unstimulated MDMs condition, with the exception of CXCL10 that was increased in the presence of the lowest sECM concentration. When MDMs were simultaneously exposed to ECM and TNFα, only the expression of the anti-inflammatory molecule CD206 was observed.

DISCUSSION & CONCLUSIONS: Fibroblast-derived ECM support MDMs basal or anti-inflammatory phenotype, even if obtained from different donors. Moreover, the presence of ECM seems to attenuate the response to a pro-inflammatory stimulus. Overall, our study shows that cells-own ECM do not activate macrophages, warranting the representation of the physiologic skin phenotype although might have a role in an inflammatory microenvironment.

ACKNOWLEDGEMENTS: ERC-2016-COG-726061 and PD/BD/14301/2018

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Keywords: Immunity / immunomodulation / macrophage, Skin

OP-204

Magnetic mesoporous silica nanoparticles for Combined Antibiofilm Therapy

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INTRODUCTION: A crucial challenge to face in the treatment of biofilm-associated infection is the ability of bacteria to develop resistance to traditional antimicrobial therapies based on the administration of antibiotics alone [1]. This study aims to apply magnetic hyperthermia together with controlled antibiotic delivery from a unique magnetic-responsive nanocarrier based on mesoporous silica nanoparticles (MSNs) for a combination therapy against biofilm.

METHODS: Levofloxacin-loaded MSNs were coated with a thermosensitive poly-N-isopropylacrylamide (PNIPAM) polymer with the ability to undergo a hydrophilic-to-hydrophobic (linear-to-globular) phase transition at a temperature between 40–43 °C. The outermost surface of the polymer-coated nanosystem was decorated with superparamagnetic iron oxide nanoparticles (SPIONs), which were obtained by thermal decomposition method [2]. The SPIONs served as hot spots since they are able to generate heat upon application of an alternating magnetic field (AMF), reaching the temperature needed to induce the polymeric conformational change and triggering pore uncapping and antibiotic cargo release. The antibacterial efficacy of the nanosystem was in vitro evaluated in mature *E. coli* biofilms.

RESULTS: Deep characterization of the nanosystem after the different stages of the synthetic procedure demonstrated the successful assembly of the different functional building blocks into the mesoporous silica-based nanoplatform. The microbiological assays indicated that exposure of *E. coli* biofilms to 200 µg/mL of the nanosystem and the application of an AMF (202 kHz, 30 mT) decreased the number of viable bacteria by 4 log₁₀ units compared to the control.

DISCUSSION & CONCLUSIONS: The clever design of the nanosystem, with SPIONs decorating the outermost surface, allows the close contact of the magnetic elements with the target, i.e., the biofilm, improving the bactericidal effect because the heating is directly on the biofilm zone and is not shielded by the silica structure. In addition, the slight antimicrobial activity of the SPIONs themselves is enhanced by the developed nanosystem. This proof-of-concept based on the combination of magnetic-hyperthermia therapy and thermo-responsive antibiotic delivery opens up promising expectations in the development of new nanotherapeutics for the management of biofilm-associated infections.

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Keywords: Nanomaterials (inc graphene), Drug delivery

OP-205

Fast acting Nitric Oxide Releasing Polymer Coatings to Protect Against Viral and Bacterial Infection

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INTRODUCTION: Healthcare-associated infections (HCAI) are a significant cause of morbidity and mortality and represent a substantial economic burden by increasing treatment and length of hospital stay. Along with cleaning and disinfection, the application of antimicrobial coatings can be a powerful tool in infection control, mitigating outbreaks of HCAIs.

Nitric oxide (NO) exhibits broad spectrum antimicrobial activity against a variety of pathogens, including bacteria and viruses, and as such has numerous uses in medical applications and therapeutics. However, as NO is a gas, the use of a NO donor is required to enable the controlled delivery of NO from materials. S-nitroso-N-acetylpenicillamine (SNAP) is a synthetic, stable NO donor that releases NO in the presence of heat, light or metal ions. This work reports the fabrication of polymer coatings containing SNAP for antibacterial/antiviral applications.

METHODS: SNAP was synthesised and incorporated into a medical grade polyurethane. To determine the suitability of the coatings for medical applications, a comprehensive study on NO payloads in different solutions (broth, cell culture media, buffer) under various conditions (pH, temperature, EDTA) was determined by chemiluminescence. The antibacterial/antiviral efficacy against methicillin resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa* (PAO1) and two variants of SARS-CoV-2 (Delta and Omicron) was determined by planktonic, biofilm and plaque reduction assays respectively. Cytocompatibility of the coatings was determined by exposing L929 cells to NO containing leachate for 4 and 24h and MTT assay.

RESULTS: Optimal coatings demonstrated high NO payloads, releasing over 600 μM of NO for in excess of 48 hours in buffered solutions. In antibacterial tests, coatings demonstrated up to 4 log reduction against MRSA within 24 hours and up to 1 log reduction of PAO1. Coatings also completely inhibited biofilm formation. In virology experiments with both variants of SARS-CoV-2, a 1 log reduction was observed after as little as 10 min contact time and up to 4 log reduction was observed within 4 hours. The coating also demonstrated excellent cytocompatibility with over 90 % cell viability observed after 24 hours.

DISCUSSION & CONCLUSIONS: The NO releasing coating developed in this work delivers a controlled and sustained payload of NO that demonstrates excellent antibacterial and antiviral activity in short contact times. These dual antimicrobial coatings show significant promise and could be used in healthcare settings and on PPE to mitigate transmission of pathogens commonly associated with HCAIs.

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Keywords: Biomaterials,

OP-206

3D bioprinting of a hypoxia-gradient for generating heterogenous cartilage scaffolds

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INTRODUCTION: Heterogenous, patient-specific tailored cartilage scaffolds are needed to repair large nasal cartilage defects (after trauma or tumor removal). Recent work has shown that the phenotype of chondrocytes can be controlled by regulating their oxygen concentration. Including oxygen gradients in scaffolds offers a strategy to control chondrocytes' phenotype by activating specific pathways that lead to hyaline or hypertrophic cartilage formation. This study aimed to investigate hypoxia-related activation of differentiation pathways in nasal chondrocytes (NCs) by embedding the NCs in a collagen/tyramine hyaluronan-based (Col/HA-Tyr) hydrogel with tunable composition. The generated bioink is used to fabricate biomimetic 3D bioprinted constructs with varying pore sizes to induce the zonal cartilage organization.

METHODS: Primary human NCs were isolated from the nasal septum cartilage of patients undergoing septoplasty. Cells were cultured either in 2D or 3D aggregates (i.e., pellets) under normoxic conditions (21%-O₂), hypoxic conditions (2%-O₂), or in a normoxic environment supplemented with hypoxia-inducing compound (21%-O₂ + 1mM DMOG). The effect of oxygen tension on chondrogenesis was determined with histological assessments of matrix deposition (by Alcian Blue, AB, or Safranin-O), protein identification by western blot, and evaluated gene expression through RT-qPCR.

NCs were embedded into the Col/HA-Tyr (0.25%/1.5%) hydrogel with Ruthenium/Sodium Perfulfate as a photoinitiator for the bioprinting part. Constructs were printed using a pneumatic bioprinter (REGENHU, Villaz-St-Pierre, Switzerland) and crosslinked using visible light afterward. Printed constructs are being analyzed for their viability and chondrogenic potential.

RESULTS: NCs cultured in hypoxia and those conditioned with DMOG maintained their native spheroidal shape and were positive for AB stainings. Conversely, NCs cultured in normoxic conditions showed an elongated-fibroblastic morphology and a limited amount of AB-positive matrix. Moreover, hypoxia-conditioned NCs exhibited statistically significant higher gene expression of the hypoxia marker PHD3 and chondrogenic markers COL2A1, ACAN, and SOX9, and lower expression of the hypertrophic cartilage markers MMP13, RUNX2, and ALPL. Similar results were obtained in 3D pellet cultures. Protein analysis revealed the abundance of hypoxia-related factors (e.g., HIF-1 α) in DMOG and hypoxia-cultured cells, which was missing in the normoxic condition.

Initial viability analysis shows the high survival of NCs in the Col/HA-Tyr-based hydrogel after printing.

DISCUSSION & CONCLUSIONS: Hypoxia positively affected NCs chondrogenic potential and supported the generation of hyaline-like cartilage tissues. Future activities will be carried out using 3D bioprinting to generate scaffolds with a controlled topology resulting in scaffold-intrinsic oxygen gradients to guide the formation of different cartilage phenotypes.

Keywords: Biofabrication, Additive manufacturing

OP-207

Influence of calcium phosphate particle incorporation approach on the calcium phosphate/hyaluronic acid composite hydrogel properties

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INTRODUCTION: As one of the regulators of cell adhesion, differentiation and proliferation in extracellular matrix, hyaluronic acid (HA) has attracted extended interest, specifically in the hydrogel synthesis. To overcome poor mechanical properties and rapid degradation *in vivo*, HA polymer chains can be chemically crosslinked and combined with calcium phosphate (CaP) nanoparticles. Additionally, this approach provides bone-mimetic microenvironment and promotes bone regeneration. Thus, the aim of current study was to investigate the effect of CaP addition approach on the properties of HA hydrogels.

METHODS: HA hydrogels (HA_H) and two types of HA/CaP hydrogels were prepared, where in one case CaP nanoparticles were added to the HA in the process of hydrogel synthesis (Mech_HA/CaP_H), but in other case CaP particles were synthesized directly in the HA solution and obtained composite material was used for the hydrogel (Synt_HA/CaP_H) preparation. The resulting reaction mixtures were crosslinked with 1,4- butanediol diglycidyl ether for 22 h and neutralized in 0.9% NaCl solution for 48 h. Swelling, gel fraction, rheology, molecular structure, microstructure, and *in vitro* biocompatibility were assessed.

RESULTS: Addition of CaP and the approach used for CaP particle incorporation into the HA matrix, increased the crosslinking degree by ~18% for Mech_HA/CaP_H and ~10% for Synt_HA/CaP_H. Additionally, storage modulus was extracted from the frequency sweeps at 1 Hz and for Mech_HA/CaP_H it was considerably higher (42±9 kPa) than for Synt_HA/CaP_H (21±2 kPa) and HA_H (2±0.4 kPa). Furthermore, CaP particles were homogenously distributed within the HA matrix for Synt_HA/CaP_H, while for Mech_HA/CaP_H CaP agglomerates were formed (40-250µm). All samples contained 90% of interconnected pores with average diameters from 100 - 400µm. Cytotoxicity of hydrogel extracts was evaluated using three cell lines (NIH 3T3, L929 mouse fibroblasts and human osteoblasts MG-63). Even though 3T3 cell viability for all samples was higher than 70%, the highest cell viability (>80%) was found for HA_H samples.

DISCUSSION & CONCLUSIONS: It was found that the properties of hydrogels strongly depend on the CaP presence, and on the approach of CaP particle incorporation into the HA matrix. Moreover, obtained *in vitro* results revealed that the effects on cell viability significantly varied between the cell lines (3T3, L929, and MG-63), emphasizing the importance of the cell line choice for the biomaterial cytotoxicity assessment.

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Keywords: Composite materials, Hydrogels and injectable systems

OP-208

UV-Crosslinked Human Platelet Lysate - a New Generation of Hydrogels for Bone Regeneration

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INTRODUCTION: Bone implants for critical-size bone defects often face challenges during regeneration, consequently influencing the patient's functional outcome. Bio-integration of the artificial implant is complicated with increasing defect size and the influence on biological processes, such as the transport of essential nutrients, biological factors, and cell ingrowth. [1] Therefore, this study aimed to develop bio-interactive hydrogels to potentially improve osseointegration of 3D-printed bone substitute scaffolds. Human platelet lysate (PL) was chosen as the main building block due to its cell-instructive and regenerative potential regarding bone regeneration and wound healing. However, the PL exhibits weak mechanical properties, thus, efforts were made to develop photopolymerizable derivatives.

METHODS: PL was modified with photopolymerizable groups (e.g., allyl glycidyl ether (PLAGE)) and compared to gelatin benchmark systems. Thiol ene chemistry was employed, and hydrogel characterization was performed via an extensive in situ photorheology, swelling and in vitro study.

RESULTS: A library of photocrosslinkable PL derivatives was synthesized, and crosslinking was successfully performed. Hydrogel formation occurred with and without a thiol in the hydrogel formulation. PL is mainly based on serum albumin, with cysteine as the most abundant amino acid, suggesting that thiol groups in the protein backbone support gel formation.

DISCUSSION & CONCLUSIONS: A versatile hydrogel platform based on PL, with the potential for improved osseointegration of artificial bone implants, is presented. Gelation without a thiol has to be highlighted, which suggests that thiols in the PL backbone support crosslinking. For the 3D fabrication of bio-integration promoting hydrogel constructs, a suitable system is to be found by variation of parameters (modification, crosslinker, gel content, e.g.).

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Keywords: Polymers - natural / synthetic / responsive, Bone and bone disorders (osteoporosis etc)

OP-209

GreenBone - advanced approaches in bone regeneration with immune-instructive biomaterials

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INTRODUCTION: Age-driven immune signals cause a state of chronic low-grade inflammation and in consequence affects bone healing and causes challenges for clinicians when repairing critical-sized bone defects in elderly patients. The aim In this study, we functionalized Poly(L-lactide-co-ε-caprolactone) PLCA scaffolds with RG-I (Rhamnogalacturonan-I) to investigate their ability to modulate inflammation in vitro and in vivo, as well as to promote bone regeneration in an aged rodent model.

METHODS: PCLA RG-I and PCLA (control) were tested in vitro, using human immune cells and in vivo using subcutaneous rat model. Cell response was tested using real-time PCR. For in vivo study, PCLA RG-I and control, PLCA were implanted subcutaneously into female Wistar rats and scaffolds were distributed randomly. The bone regeneration properties were tested with calvaria critical-size model in elderly (10 months) Wistar rats. Harvested samples were analysed for inflammatory markers.

RESULTS: The cell response on PCLA RG-I showed significant increase of anti-inflammatory gene and decrease of pro-inflammatory genes expression. The subcutaneous model confirmed in vitro results. In addition, there was 3 folds increase of anti-inflammatory gene expression. The histology showed sparse inflammatory capsule around PCLA RG-I scaffolds after 4 days and 4 weeks. The in vivo results in elderly rat calvaria model showed increase of osteogenic markers and decrease of proinflammatory markers and RANKL, compared to control. The mean percentage of bone volume/tissue volume in the defect with RG-I scaffold was significantly greater than the defect with control.

DISCUSSION & CONCLUSIONS: We have shown that PLCA scaffolds functionalized with RG-I with higher amount of galactose play a role in the modulation of inflammatory responses both in vitro and in vivo subcutaneously and promote the initiation of bone formation in a critical-sized bone defect of an aged rodent.

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Keywords: Biomaterials, Nanomaterials (inc graphene)

OP-210

Smart Biomimetic Scaffold Fostering A Combined Activation Of Teno-differentiation And Immunomodulation

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INTRODUCTION: In tendon tissue engineering, much attention has been given to develop innovative implantable scaffolds to overcome the limited regenerative ability of tendons [1,2,3]. In this context, this research aimed at designing 3D tendon biomimetic scaffolds able to establish a tailored microenvironment instructing amniotic epithelial stem cells (AECs) towards teno-differentiation and immunomodulatory paracrine actions tested on activated THP-1 macrophages.

METHODS: 3D tendon biomimetic PLGA scaffolds with highly aligned fibers were fabricated and compared for their structure and mechanical properties to the fleeces used as control. AECs' teno-differentiative potential was assessed analyzing the gene and protein expressions of tendon-related markers (SCX, COL1, and TNMD) after 48h of culture. The scaffold-induced AEC immune response was analyzed by recording its direct influence on IL-10 and IL-12 cell expression as well as on cell paracrine immunomodulatory activities assessing the release profile of 40 immunomodulatory chemokines/ILs (Immunomodulatory Antibody Array Membrane) in AEC-derived CM. Finally, CM's biological effect was tested on the activated human THP-1 macrophages determining the release profile of IL-6.

RESULTS: The obtained 3D scaffolds closely resemble native tendon tissue structurally and biomechanically. 3D scaffolds were able to boost AEC's teno-differentiation upregulating tendon-related genes (SCX, COL1 and TNMD) and TNMD protein expression, compared to fleeces ($p < 0.05$) after 48h of culture. Additionally, 3D scaffolds prompted AECs' immunomodulatory ability upregulating anti-inflammatory IL-10 with a favorable IL-10/IL-12 ratio compared to fleeces ($p < 0.05$). Moreover, AEC derived-CM showed that 3D scaffolds enhanced the release of several immunomodulatory molecules including IL-10, RANTES, PDGF-BB, which exhibit even an important role in tendon regeneration. The biological effect of 3D scaffolds derived CM was confirmed on activated THP-1 macrophages by increasing IL-6 release.

DISCUSSION & CONCLUSIONS: The fabricated 3D tendon biomimetic scaffolds, in terms of architecture and biomechanics, were able to establish a tailored microenvironment instructing the teno-differentiation of AECs genotypically and phenotypically. Moreover, the 3D scaffolds fostered the immune-AECs activities upregulating anti-inflammatory cytokine expression as well as the release of immunomodulatory molecules in CM able to modulate the biological activity of macrophages. Altogether, the fabricated 3D scaffolds stand as promising candidate for tendon regeneration.

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Keywords: Biomaterials, Immunity / immunomodulation / macrophage

OP-211

Scale-Up of Octacalcium Phosphate via Hydrolysis Route: Effect on Physico-Chemical Characteristics and In-Vitro Cytocompatibility with Bone Marrow-Derived Mesenchymal Stem Cells

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INTRODUCTION: Octacalcium phosphate (OCP, $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$) has kept the interest of the scientific community ever since it was postulated to be a potential precursor in bone formation. The basis of this hypothesis lies in its intrinsic structure and prospective ability to induce bone regeneration. However, obtaining a large quantity of OCP remains a significant obstacle, and its optimization remains important in order to compare its attributes among a set of characterization methods and potential clinical uses.

METHODS: OCP was synthesized through hydrolysis of α -tricalcium phosphate (α -TCP), and the synthesis was scaled-up tenfold and hundredfold (100 mg $\rightarrow$ 10 g). The phase and chemical composition were analyzed using X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR) and Raman spectroscopy. The morphology of powders was determined by a scanning electron microscope (SEM). In vitro cytocompatibility was tested using bone marrow-derived mesenchymal stem cells (hBMSCs). hBMSCs were seeded in a defined concentration (2x10⁴ cells/well) and cultivated in direct contact with OCP powders for three days. The viability of the cells was assessed by means of their metabolic activity at 24h, 48h and 72h. The morphology of cells, after the direct contact with specimens, was evaluated by immunofluorescent imaging (IF).

RESULTS: Throughout all the scale-up levels, characteristic OCP crystallographic patterns were detected (maxima at 4.7, doublet at 9.4 and 9.8 2 θ degrees). FT-IR bands characteristic of ν_3 PO₄³⁻ stretching, as well as ν_4 PO₄³⁻, ν_1 PO₄³⁻ and HPO₄²⁻ stretching, which correspond to the OCP structure, were confirmed. SEM micrographs indicated that the powders had distinctive plate-like morphology of OCP. Specimens of each scale-up proven to be cytocompatible with hBMSC, as the metabolic activity of the cells was comparable to the gold standard control (>70% viability). Furthermore, once the powders were cultivated in direct contact with cells, they showed the potential to undergo cell internalization.

DISCUSSION & CONCLUSIONS: Based on all the data acquired, obtained products exhibited all the characteristics of OCP, as well as the plate-like morphology of crystals. Bearing in mind that the precedent of the biomaterial for bone tissue engineering is to provide migration and proliferation of cells, the presented in vitro findings have been promising for the further scale-up. Additionally, the potential of obtaining high quantities of OCP has been authenticated.

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Keywords: Scaling up & manufacturing, Multipotent (mesenchymal) stem cells

OP-212

Development of a custom-made 3D-printed bone substitute for critical-size bone defect repair

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INTRODUCTION: The reconstruction of critical-size bone defects remains challenging, with autogenous bone grafting still considered the gold standard treatment despite limitations such as shortage of supply and donor site morbidity. Bone tissue engineering holds much promise to develop new bone substitutes, which can offer safety, cost-effectiveness, and efficacy. This study aims to validate the effectiveness of a bioengineered 3D printed scaffold, with controllable micro-architecture, made from the bioabsorbable polymer polycaprolactone (PCL) that has been functionalised with plasma polymerised poly-(ethyl acrylate)(p-PEA), Fibronectin (FN) and Bone Morphogenic Protein (BMP), for bone repair.

METHODS: The surface of scaffolds was characterized by AFM, water contact angle measurements, XPS, and quantification of protein adsorbed using bicinchoninic acid and ELISA. The viability of MSCs on the scaffold was assessed using fluorescent staining and Alamar blue assays. QPCR, von Kossa and Alizarin red stains were conducted to assess the osteogenic capacity of scaffolds. Lastly, nanovibrational stimulation was utilized to pre-condition the MSCs to osteogenesis prior to seeding on the scaffold to see if differentiation could be further enhanced.

RESULTS: The hydrophilicity of PCL was improved by functionalising its surface with the PEA and FN, and the quantity of FN and BMPs adsorption on the coated scaffold was significantly higher than the uncoated PCL scaffolds. The bioactivity of scaffolds was significantly increased on coated scaffolds with extremely larger numbers of cells attached and with higher metabolic activity as compared to control. Moreover, the osteogenic activity of coated scaffold was validated with notable upregulation of osteogenesis markers both by direct seeding of naïve MSCs onto the scaffolds and, even more so, with MSC pre-conditioning using nanovibrational stimulation.

DISCUSSION & CONCLUSIONS: the functionalized PCL surface (PEA+FN+BMPs) showed promising biocompatibility and osteogenic potential in vitro. Cell preconditioning is also indicated as a useful step in a combined cell-device regenerative therapy approach.

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Keywords: 3D printing and bioprinting, Differentiation

OP-213

Determining the function of genes associated with the genetic risk of catastrophic fracture in Thoroughbred racehorses

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INTRODUCTION: Fractures in Thoroughbred racehorses can be caused by bone overloading, this is in contrast with fractures that are sustained due to trauma. Bone over loading fractures often occur on the racecourse and are the leading reason for euthanasia. Previous research isolated equine fibroblasts and used a polygenic scoring system to assign a risk category.

METHODS: Using this system three horses that were identified to be at the highest and lowest risk were used to generate induced pluripotent stem cells (iPSCs). These were differentiated into osteoblasts using established methods then used in global RNA sequencing. Two fracture cases and two controls were also analysed using whole genome sequencing (WGS).

To screen differentially expressed genes that may have a novel role in bone formation, gene expression in human osteosarcoma SAOS2 cells cultured under basal and osteogenic culture conditions was analysed using qPCR.

To determine the role of these candidate genes over and under expression studies were performed and effects on osteoblast associated genes, matrix mineralisation and hydroxyapatite deposition were measured.

RESULTS: RNA sequencing produced 112 differentially expressed genes. Further analysis identified three main subgroups; 37 genes that were linked to bone function or formation processes, 27 genes that were unannotated and 48 genes that had no known links to bone function or formations pathways or processes. Pathways that are associated with many bone diseases such as the ERK signalling pathway were also identified within this cohort using gene ontology. WGS revealed that one of the differentially expressed genes, KIZ, contained an exonic variant.

Of the genes that had no known role in bone function or formation 16 genes were carried forwards. Candidate genes were identified based on expression in other musculoskeletal tissues and if there was a role in biological processes/pathways that have are involved in bone/fracture. Of these candidate genes eight are expressed at higher levels in low-risk osteoblasts and eight are expressed at higher levels in high-risk osteoblasts. Seven of these genes were not expressed at detectable levels in either culture condition. Five genes (including KIZ) exhibited increases in expression following osteogenic culture. Four genes showed a decrease after osteogenic culture, one of which was LGALS1. Modulation of the levels of LGALS and KIZ was performed and analysis is ongoing.

DISCUSSION & CONCLUSIONS: This study demonstrates the application of a genome-wide polygenic risk score to establish an in vitro cell system to identify novel genes involved in fracture risk in horses.

Keywords: Bone and bone disorders (osteoporosis etc), Veterinary

OP-214

Cellular density and mechanical stimulation affects morphology of encapsulated equine tendon and endothelial cells in modified gelatin

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INTRODUCTION: Both in human and equine athletes, tendons function close to their physiological limits and are therefore prone to develop injuries. The human Achilles tendon and the equine superficial flexor tendon are analogous in function and structure and are both frequently injured during high-intensity exercises. Currently, treatment options are limited and fail to restore tendon's original structure. In order to study potential new treatments without the confounding factors associated with *in vivo* trials, a representative *in vitro* tendinopathy model is mandatory(1). As the native extracellular tendon matrix consists mainly of water and collagen type I, gelatin-based hydrogels are ideal candidates to mimic the natural tendon environment. Hydrogels are able to retain large amounts of water while gelatin, extracted from collagen, can be chemically modified to tune its mechanical properties and it contains natural integrin binding sites, essential for cell adhesion.

METHODS: In this study, equine tendon and endothelial cells were encapsulated in bovine skin collagen (2mg/mL), methacrylated gelatin (gel-MA, 65% DS) and norbornene-functionalized gelatin (gel-NB, 55% DS)(2,3). Besides scaffold composition, the influence of cellular density on cell proliferation, viability, and morphology was investigated. Additionally, cells were mechanically stimulated 30min. per day (0.5Hz, 5% stretch) to mimic *in vivo* trotting of a horse.

RESULTS: Viability only exceeded 70% for both cell types in all scaffolds when encapsulated at high density (5×10^6 cells/mL) regardless mechanical stimulation. Cell shape was evaluated by actin staining and determination of nucleus' circularity and aspect ratio, being major indications for elongation. Without mechanical stimulation, at 5×10^6 cells/mL, the cells elongated (high AR and low circularity) significantly more when compared to lower densities. Tendon cells elongated more in gelNB55 compared to gelMA65. In response to the uniaxial tension, endothelial cells encapsulated at high density in gelNB elongated parallel to the direction of the strain.

DISCUSSION & CONCLUSIONS: When developing *in vitro* models, cellular density should be investigated as cell-cell contact is a critical success factor. In order to maximize cellular responses, a concentration of 5×10^6 cells/mL in gelNB55 was superior for both equine tendon and endothelial cells. Mechanical stimulation did not influence viability, yet improved cell morphology. These data strengthen our knowledge on the interaction between natural biomaterials and tendon resident cells, which is essential to establish a representative tendinopathy model.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Hydrogels and injectable systems

OP-215

Canine Mesenchymal Stem/Stromal Cells as novel tools for combating antimicrobial resistance

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INTRODUCTION: Mesenchymal stem/stromal cells (MSCs) have been used for decades in regenerative therapies to treat musculoskeletal injuries. MSCs are obtained from diverse body tissues, resulting in preparations with variable properties that can be enhanced upon cell activation when exposed to stimulants. MSCs are characterized by the expression of MSC markers (CD73, CD90 and CD105) and trilineage cell differentiation into adipocytes, chondrocytes and osteocytes. Recently, MSCs are being investigated as a novel tool to combat infection, namely those caused by bacteria displaying antimicrobial resistance (AMR). MSCs' capacity to inhibit bacterial growth is based on their combined ability to secrete antimicrobials and immunomodulatory cytokines. Unresolved AMR infections in dogs pose an alarming threat to companion pets and owners sharing the same environment, and new strategies are needed to tackle this global threat.

This project aims to isolate canine MSCs from different tissue origins to identify cells with robust antimicrobial properties.

METHODS: Canine adipose (AT) and endometrial (EM) tissues were obtained from reproductive tract of dogs (n=3) undergoing neutering. AT- and EM-MSCs were isolated and characterized by trilineage cell differentiation and qPCR of MSC markers and genes measured upon stimulation with lipopolysaccharide (LPS). To assess MSCs' antibacterial activity, *Staphylococcus pseudintermedius* (3×10^6 CFU/ml) was inoculated in conditioned medium (CM) prepared by growing MSCs in serum- and antibiotic-free medium for 48 hours. Bacterial growth was assessed by optical density at 600nm.

RESULTS: MSC characterization showed that gene expression levels of MSC markers CD73, CD90 and CD105, and cell differentiation into adipocytes, chondrocytes and osteocytes was similar for AT and EM-MSCs. Both cell types were responsive to the bacterial wall-component LPS resulting in upregulation of the cytokine IL8, and immune cell chemoattractants CCL2 and CCL5 ($p < 0.05$). There were no differences in levels of expression by AT- and EM-MSCs, except for IL8 which was significantly higher for EM-MSCs compared to AT-MSCs upon stimulation ($p < 0.0001$). In addition to these findings, our preliminary results indicated that conditioned medium (CM) from AT- and EM-MSCs cultures attenuated the growth of canine multi-drug resistant bacterium *S. pseudintermedius*, with the two cell types showing a similar effect.

DISCUSSION & CONCLUSIONS: The results indicate that canine EM, which can be sourced from routine neutering procedures, maybe a convenient alternative to obtaining MSCs for veterinary regenerative medicine. The anti-infective activity of AT- and EM-MSCs suggest MSCs could be a valuable tool in the combat of AMR, with relevance for canine and One-health.

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Keywords: Multipotent (mesenchymal) stem cells, Infection

OP-216

A Murine articular progenitor cell implantation and tracking model reveals that implanted cells recapitulate endochondral ossification

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INTRODUCTION: Endochondral ossification is the process by which mammalian long bones develop, repair and regenerate. This process involves the creation of cartilage tissue followed by bone. Recent experimental evidences indicate that hypertrophic chondrocytes can become osteoblasts at bone development, suggesting a chondrocyte plasticity during endochondral ossification[1]. Until now, this has not been fully studied in bone tissue engineering context. The objective of this work is to recapitulate endochondral ossification in a regenerative context and define the fate of implanted mouse progenitor cells, to further modulate this process and potentiate bone formation.

METHODS: We used multiple transgenic mouse models to isolate GFP+ and Tomato+ murine articular progenitors from 5 days-old pups[2]. Progenitor cells were characterized, seeded in gelatin scaffolds and implanted in NOD-SCID mice subcutaneous tissue, with or without rhBMP-2 to induce bone formation[3,4]. Samples were retrieved at different time points and tissue formation was analyzed by computerized tomography, histology and immunostaining to define the location and phenotype of implanted cells.

RESULTS: The isolated cells expressed fluorescent proteins depending on their cartilage differentiation marker expression. At implantation, fluorescent murine cells were cartilage in early harvesting time points. At late time points, the fluorescent cells were observed as bone cells phenotype.

DISCUSSION & CONCLUSIONS: Data indicate that gelatin scaffolds are valid mouse progenitor cell carrier material for in vivo implantation procedures, while the approach recapitulates endochondral ossification in mouse cell context.

In conclusion, data indicate that an approach compiling transgenic mouse primary articular progenitor cell extraction and their implantation in gelatin scaffolds allows the tracking of the cells and defining their fate. Implanted cells undergo endochondral ossification, including transition from cartilage to bone phenotype in in vivo context.

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Keywords: Bone and bone disorders (osteoporosis etc), Biologics and growth factors

OP-217

Resveratrol and resveralogues suppress the damaging phenotype of senescent equine tenocytes

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INTRODUCTION: The superficial digital flexor tendon (SDFT) of the horse is a highly relevant model for studying tendinopathies (1). Previous studies with cyclical loading of tendon explants have revealed that injured SDFTs produced high levels of pro-inflammatory cytokines and 10-1000-fold overexpression of matrix metalloproteinases with impaired resolving mechanisms, especially in older horses (2). Senescent cells are generated by many pathways of which produce viable cells that (a) overproduce pro-inflammatory cytokines collectively known as the Senescence Associated Secretory Phenotype (SASP) and (b) remodel matrix, which are implicated in age-related degenerative changes that predispose to tendinopathies. Corticosteroids frequently used clinically to treat tendinopathies initially block the inflammatory component of tenocyte senescence but also may cause therapy-induced senescence and interfere with tendon repair, contributing to the high re-injury rate (3). The aim of this study was therefore to use an in vitro model to investigate the effects of dexamethasone on senescence and rescue or by small molecule senescence inhibitors.

METHODS: Tendon derived cells (TDCs), isolated from the superficial digital flexor tendon (SDFT) of adult horses (11 years old, n=3) were cultured in DMEM (supplemented with 10% fetal bovine serum and 1% P/S) in a humidified incubator with 5% CO₂ at 37°C. TDCs were co-treated with resveratrol, resveralogues (V34 and V29) and 1 and 10µM dexamethasone for 48 hours and then cultured in medium without dexamethasone for a further 24, 72 and 96h. Effect of dexamethasone on cell cycle was measured by cytochemical analysis with EdU (Click-iT Plus EdU Cell Proliferation Kit, Fisher) and Ki-67 (Rabbit monoclonal to Ki67, Abcam). Senescence was confirmed by measuring of gene expression of senescence-associated markers and the development of a unique SASP at each time point with a cytokine array kit (Raybiotech).

RESULTS: Treatment with dexamethasone rapidly induces a senescent state characterised by growth arrest, the accumulation of p53 and p21 followed by p16 (p<0.01), and the development of a unique SASP in which IL-8 and MCP-1 are upregulated. Resveratrol and resveralogues (V34 and V29) protected against dexamethasone induced senescence (p<0.001). In addition, resveratrol blocked the SASP.

DISCUSSION & CONCLUSIONS: Dexamethasone at clinically relevant doses induced growth arrest in equine tenocyte cells. These data provide a mechanistic explanation for potential adverse effects of using corticosteroids for the treatment of tendinopathies. Resveratrol and resveralogues partially reverse dexamethasone-induced senescence.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Veterinary

OP-218

Comprehensive understanding of sheep as model organism: A tissue proteome atlas and a tissue eicosanoid atlas

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INTRODUCTION: Animal models are an important tool used in human and veterinary medicine for the detailed analysis of complex physiological processes. However, the translatability of results obtained in model animals to humans or an animal species of interest is often still insufficient. Sheep are a well-established large animal model, which is used in multiple fields of biomedical research. In order to achieve better comparability of the results obtained in sheep with humans or any animal species of interest a deep understanding of the model organism is required. Proteome profiling supports the investigation of expression profiles characteristic for tissues, cells and functional states. Fatty acids and their derivatives such as eicosanoids and endocannabinoids represent highly relevant lipid hormones recognized to be responsible amongst others for the regulation of pain sensation, blood coagulation, transport processes, mucus formation and inflammatory processes. Up until now, no comprehensive experimental data exist with regard to tissue proteomics and the corresponding tissue-specific background of eicosanoids. This motivated us to establish a proteome atlas and an eicosanoid atlas of tissue samples from healthy adult sheep.

METHODS: Tissue samples from brain, cartilage, heart, kidney cortex, kidney mark, liver, lung, muscle, nerve, skin, spleen and tendon from three animals (adult ewes, age 2 to 5 years) were harvested and analyzed using established protocols for proteome profiling (PMID: 34884768) and eicosanoid analysis (PMID: 33467719) based on chromatography hyphenated with high-resolution mass spectrometry.

RESULTS: In total, 4778 proteins were identified with high confidence, allowing for comparative expression profiling. The expression of metabolic enzymes, focusing on glycolysis, beta-oxidation, glutaminolysis, and lipid-processing enzymes was found to significantly differ between the tissue types. 21 fatty acids, 80 fatty acid oxidation products such as eicosanoids and 20 endocannabinoids were identified and evaluated regarding their tissue-specific expression. Again, molecular profiles discriminating the analyzed tissue types, were successfully characterized. Performing a combined analysis, we were able to relate the expression of fatty acid-modifying enzymes determined via proteomics with the corresponding lipid species as enzymatic products.

DISCUSSION & CONCLUSIONS: The presented data indicate tissue-specific metabolic activity states, which may act as powerful modulators of immune functions and regenerative processes.

REFERENCES: PMID: 34884768

PMID: 33467719

Keywords: Omics / bioinformatics and systems biology, Veterinary

OP-219

Umbilical Cord MSC Treated Osteoarthritic Sheep Display Reduced Radiographic Changes: Proteomic Biomarker Profiling of Synovial Fluids Implicate an Immunomodulatory Mechanism

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INTRODUCTION: Our group has investigated umbilical cord-derived mesenchymal stromal cells (hUC-MSCs) as a therapeutic option for osteoarthritis in a series of in vitro and in vivo studies (1–4). Here we report the structural and proteomic changes detected in an ovine model of early osteoarthritic (OA) sheep treated with hUC-MSCs.

METHODS: Medial meniscal transection in the left stifle joints of 14 female, 3–4 year old Welsh Mountain sheep was performed. Four weeks post-surgery, 107 hUC-MSCs (pooled from 3 donors) in 50µL PBS (n=7) or PBS alone (n=7, vehicle control group) were injected into the transected joints. Joints and synovial fluids (SFs) were harvested 12-weeks post-surgery. Scoring of structural changes were performed via a macroscopic OA score, Kellgren-Lawrence score (X-ray), sMOAKS (whole joint on MRI) and pyKNEEr (cartilage thickness on MRI), and cartilage histology scores. Proteomic changes in SFs were assessed via Label-Free proteomic and Ingenuity Pathway analyses.

RESULTS: Kellgren-Lawrence X-ray scores in joints treated with hUC-MSCs were significantly improved (2.0 ± 0) cf. control (3.0 ± 0 ; $p=0.028$). No significant differences in macroscopic, sMOAKS, pyKNEEr or histologic scores were observed between the treatment groups, although trends for each observation indicated lower levels of structural disease in sheep receiving hUC-MSCs. Proteomic and bioinformatic analyses found 58 proteins differentially abundant between treated and untreated animals (>1.2 FC). Several pathways associated with immune cell movement were predicted to be activated, whereas cellular infiltration by leukocytes and myeloid cells were predicted to be inhibited in treated sheep. Interestingly, death of connective tissue cells was also predicted to be significantly inhibited in treated animals.

DISCUSSION & CONCLUSIONS: Here we report reduced structural OA burden in sheep treated with hUC-MSCs cf. vehicle controls and proteomic and bioinformatic analyses indicate that immune cell movement and infiltration is modified in the SF of treated animals. There remains uncertainty regarding the mechanism of hUC-MSC therapies. Future studies investigating the specific tissue responses to hUC-MSC treatment are needed to fully unpick therapeutic action and to drive forward clinical translation of the approach.

ACKNOWLEDGEMENTS: Versus Arthritis Tissue Engineering and Regenerative Therapies Centre.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Cell therapy

OP-220

Development of a Physiologically Relevant Human Small Intestine Model

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INTRODUCTION: Small intestine plays a major role in digestion and absorption of nutrients as well as in drug metabolism. For decades, in vitro small intestinal models relied on culturing cell lines on cell inserts to form a polarized barrier. However, these barriers are not representative of the small intestine as they exhibit abnormally high transepithelial electrical resistance (TEER) and fail to mimic the complex tissue architecture. In this study, we aimed to establish a 3D reconstructed intestine model that represents the characteristics of human small intestine.

METHODS: Intestinal epithelial cell lines or primary duodenum derived epithelial organoids were grown on the surface of either an empty 3D matrix in the presence of primary duodenum fibroblast supernatant to investigate the effect of paracrine factors, or on the surface of a 3D matrix populated with primary duodenum fibroblasts to investigate the effect of living cell-cell crosstalk. Models were kept in culture for 2-3 weeks to allow barrier formation. Cell lines and organoids cultured on cell inserts without the 3D matrix were used as controls. Chemokine secretion was assessed in culture supernatants.

RESULTS: Polarized epithelial barriers exhibited significantly reduced TEER both in the presence of paracrine factors and the fibroblasts. Furthermore, organoid models in the presence of fibroblasts showed matrix remodeling and formed structures resembling villi, leading to a human intestine model with the characteristics of small intestine. Differential chemokine secretion was observed between the models.

DISCUSSION & CONCLUSIONS: The 3D configuration of the small intestinal model revealed the role of fibroblasts in shaping the barrier properties and led to a more physiologically relevant model in terms of tissue architecture, demonstrating a more accurate system for studies on human small intestine.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Differentiation

OP-221

Mass production of functional human cardioids using in-air generated thin-shelled microcapsules

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INTRODUCTION: Organized cellular spheroids and organoids are microtissues that hold great potential for tissue engineering and drug testing purposes. However, these microtissues are currently produced using batch-process techniques that associate with artisan, labor-intensive, and low throughput production processes. This hinders the clinical and industrial application of these microtissues. Continuous tissue production techniques such as microfluidics are anticipated to enable the large scale production of microtissues. However, current on-chip microfluidic droplet generation have unable to deliver on this promise as they operate within the slow dripping regime. A novel solution to enable scalable production of microtissues has therefore remained wanted.

METHODS: A novel triple-jet in-air microfluidic platform was developed for the ultra-high throughput production of hollow, thin-shelled, hydrogel microcapsules that can act as organoid forming bioreactors. Specifically, a piezo-actuated monodisperse droplet train of human pluripotent stem cell (hPSCs)-laden liquid containing calcium was collided with a liquid jet containing alginate, which was collided with a third liquid jet that contained alginate to form cell-laden core-shell microgels. Cells were induced in myocardial lineage using differentiation media. Confocal microscopy, dual fluorescent reporter cell-lines (i.e., MESP1/NKX2.5/ α -actinin), calcium flux stains, and single cell RNAsequencing was used to investigate the function and phenotype of mass produced cardiospheres.

RESULTS: Microencapsulating hPSCs in hollow microgels using IAMF at jetting speeds allowed for more than 420 fold higher production rates than conventional droplet microfluidic platforms. The hollow microgels allowed for autonomous self-organization of hPSC spheroids and enabled long-term stable 3D suspension culture with maintained pluripotency. Uniquely, IAMF microcapsules provided a lumenogenic microenvironment with near 100% efficient cavitation of hPSC spheroids resembling embryoid body's rosette-stage; autonomous self-organization with polarized cytoarchitecture was achieved without any added growth factors. Upon chemical stimulation, hPSC-derived spheroids underwent cardiomyogenic differentiation effectively resulting in mass production of a homogeneous and functional (i.e., contracting) cardiospheres that were responsive to external electrical stimulation. Single cell RNAseq revealed that mass produced cardiospheres obtained a cardiomyocyte phenotype that was near-indistinguishable as conventional low-throughput produced cardiospheres.

DISCUSSION & CONCLUSIONS: In short, we report on an innovative method that enables efficient production of high-quality organized spheroids, embryoid bodies, and organoids in a cytocompatible, ultra-high-throughput, and clean manner, thereby driving clinical and industrial adaption of stem cell technology in application areas such as tissue engineering, drug testing, and cultured meat.

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Keywords: Enabling technologies, Scaling up & manufacturing

OP-222

3D spheroids co-culture of human adipose stem/stromal cells and endothelial cells for bone tissue engineering optimization

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INTRODUCTION: Vascularization remains as a technological bottleneck in tissue engineering. Scaffold-free technologies, in particular tissue spheroids, hold advantages mainly based on their self production of extracellular matrix components. Recently, our research team showed distinct levels of cartilage maturation from adipose stem/stromal cells (ASC) spheroids (Kronemberger et al., 2020; Côrtes et al., 2021). However, hypertrophic cartilage derived from ASC spheroids was not able to reach a mature bone phenotype (the presence of hydroxyapatite crystals in extracellular matrix). In this study, the objective was to establish a protocol of ASC spheroids co-cultivated with endothelial cells (EA.hy926) to mimic a tissue microenvironment that favors the late stage of bone maturation, providing a vascularized construct.

METHODS: ASC and EA.hy926 cells were monitored by the expression of typical cell surface markers evaluated using flow cytometry. ASC/EA.hy926 spheroids were formed and cultivated in micro-molded nonadhesive hydrogels with 81 circular recesses fabricated according to manufacturer's recommendations (MicroTissues 3D Petri Dish). A range of cell concentration and proportions was tested and the resulting spheroids were induced to osteogenic pathway (Kronemberger et al., 2020) and characterized by CellTiter-Glo® 3D Cell Viability Assay (Promega), diameter measurements, histological analysis (hematoxylin & eosin and alizarin red stainings) during 2, 3 and 5 weeks.

RESULTS: ASC showed positivity for CD105, CD73, CD90 and CD34 and EA.hy926 cells for CD31, as expected. The diameter measurements revealed the proportion of 4:1 (ASC/EA.hy926) was the most promising in terms of size (333,38±18,52) and shape (sphericity close to 1). Viability assays revealed no significant statistical difference during ASC/EA.hy926 spheroids culture. Hematoxylin & eosin staining revealed a preferred location of ASC inside and EA.hy926 on the surface of spheroids, to be confirmed by the immunostaining of CD90 e CD31, respectively. ASC/EA.hy926 spheroids induced to osteogenesis showed extracellular calcium depots at week 5 revealed by alizarin red staining.

DISCUSSION & CONCLUSIONS: ASC/EA.hy926 spheroids will be characterized for gene expression and analysis of extracellular matrix molecules involved in the osteogenesis pathway, in addition to the dosage of cytokines and growth factors in the culture supernatant. Transmission electron microscopy for the detection of hydroxyapatite crystals and sprouting assays are the next step to attest the ASC/EA.hy926 spheroids functionality for bone tissue engineering.

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Keywords: Bone and bone disorders (osteoporosis etc), Multipotent (mesenchymal) stem cells

OP-223

A novel 3D-bioprinted patient-specific biomimetic bone organoid to model osteogenesis imperfecta

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INTRODUCTION: Osteogenesis imperfecta (OI) is a rare genetic heterogeneous connective tissue disorder characterized by bone fragility. Despite extensive molecular studies in animal models, the disease pathomechanisms are not yet fully understood, thereby impairing the development of targeted pharmacological therapies. Here, we propose a novel approach to model OI *in vitro* using a 3D bioprinted patient-specific biomimetic bone organoid.

METHODS: Osteoblasts were isolated from a 3-year-old female with *FKBP10*-OI and a metabolically healthy 15-year-old male. Cells were encapsulated in alginate-gelatin-graphene oxide hydrogels and extrusion 3D-bioprinted [1]. Healthy and OI bone organoids were cultured in compression bioreactors for 8 weeks and subjected to uniaxial cyclic compressive loading. After 4 weeks, organoid surfaces were reseeded with osteoblasts from the same donor. Organoid maturation was investigated by weekly time-lapsed micro-computed tomography (micro-CT) and mechanical testing. Endpoint histology, and immunohistochemistry enabled characterization of cellular phenotypes and mineralized matrix formation, whilst Picrosirius Red staining with Polarized Light Microscopy (PLM) informed on collagen fibre maturation [1].

RESULTS: A significantly higher average tissue mineral density was found in OI organoids after 35, 42 and 49 days of culture (127.40 ± 4.52 , 137.77 ± 4.48 , 146.91 ± 3.19 mg HA/cm³, respectively) compared to healthy controls (120.24 ± 1.49 , 129.84 ± 2.46 , 139.48 ± 3.89 mg HA/cm³) ($p < 0.05$). Endpoint compression testing revealed an average stiffness of 0.83 ± 0.49 N/mm for OI samples and 0.975 ± 0.13 N/mm for controls. Reseeded cells adopted fibroblastic morphologies, while the cells encapsulated in the hydrogel at day 0 presented with a more dendritic osteocyte-like morphology. Picrosirius Red and Alizarin Red S staining revealed collagen deposition and mineral nodule formation on filament surfaces and pores colocalized with osteoblastic cells. PLM revealed a larger percentage of immature collagen fibres in OI organoids, compared to a more mature fibre distribution in healthy controls.

DISCUSSION & CONCLUSIONS: We successfully established a 3D *in vitro* patient-specific bone model for OI. *FKBP10*-OI osteoblasts were able to produce denser mineral than healthy controls, consistent with findings of OI bone biopsies [2]. However, no significant difference was found in stiffness between groups at endpoint. We demonstrated distinct phenotypic differences in OI and healthy control bone organoids in terms of collagen fibre maturity. The established bone organoid may become a powerful tool to study pathomechanisms for numerous OI mutations and facilitate the development and testing of advanced therapies on a patient-specific level.

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Keywords: Disease models, Bone and bone disorders (osteoporosis etc)

OP-224

Directing the fusion, growth and remodeling of cellular microtissues to engineer anisotropic soft tissues

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INTRODUCTION: Regeneration of articular cartilage (AC) requires engineered grafts that mimic the phenotype and functionality of the native tissue. Despite sustained efforts, engineering biomimetic AC remains a challenge. The problems encountered with the use of classical tissue engineering approaches have motivated the development of scaffold-free strategies, where cellular aggregates, microtissues or organoids can be used as building blocks in the biofabrication of scaled-up constructs. Relatively few studies have explored the capacity of such microtissues to fuse *in vitro* and generate functional AC grafts (1). The aim of this work is to better understand the mechanisms behind cartilage microtissue fusion.

METHODS: Human bone marrow mesenchymal stem cells (MSCs) derived cartilage microtissues were fabricated in micro-moulds as described previously (2). After 2, 4 and 7 days of culture, the microtissues were harvested to investigate their capacity to fuse and form a scaled-up AC graft. After 28 days in chondrogenic induced media (CDM), chondrogenic differentiation in the fused graft was assessed by histology, immunohistochemistry, biochemical assays, and RT-qPCR. Next, the microtissues at day 2 of maturation were fused to regenerate a centimeter-sized (10x1 mm) tissue and cultured for 6 weeks in CDM, in dynamic and static conditions, at different oxygen tensions (20% and 5 %).

RESULTS: Constructs generated by the fusion of cartilage microtissues contained higher levels of glycosaminoglycans (GAGs) after 28 days in CDM. Less mature microtissues (removed from micro-moulds at day 2 and 4) possessed a stronger capacity for fusion and ultimately generated a macro-tissue containing higher levels of GAGs compared to more mature microtissues (i.e., those removed from micro-moulds at day 7). Next, we investigated the potential of microtissues to form a large-scale tissue that mimics the size of defects typically observed in human AC. In all the four conditions (static and dynamic; 5% and 20% O₂) the microtissues were able to fuse and generate a scaled-up tissue over 6 weeks of culture in CDM.

DISCUSSION & CONCLUSIONS: The final conclusions are: (1) microtissues cultured independently for relatively short periods of time (2-4 days) possess a superior capacity for fusion and generate scaled-up tissues with a more cartilage-like phenotype; (2) it is possible to engineer scaled-up scaffold-free grafts using multiple cartilage microtissues; (3) dynamic conditions don't negatively affect the capacity of microtissues to fuse. The work supports the use of microtissues to engineer scaled-up tissues for AC regeneration.

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Keywords: Cartilage / joint and arthritic conditions, Biofabrication

OP-225

Injectable self-healing hydrogel delivering microRNAs-loaded nanocarriers as a promising advanced therapy for cardiac regeneration

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INTRODUCTION: Cardiovascular diseases are the leading cause of death worldwide. Myocardial infarction (MI) causes the irreversible loss of cardiomyocytes and the formation of a dysfunctional scar tissue, leading to heart failure [1]. Recently, the delivery of microRNAs (miRNAs) able to induce direct cell reprogramming (DR) has emerged as a new option for in situ myocardial regeneration. Previously, we have demonstrated that transient transfection of human adult cardiac fibroblasts (AHCfs) with four microRNA mimics ("miRcombo") using commercial Dharmafect (DF) triggers DR into induced cardiomyocytes (iCMs) in vitro [1]. In this work, we designed safe and efficient nanocarriers for in situ DR of AHCfs into iCMs [2] and injectable self-healing hydrogels for their local administration.

METHODS: Lipoplexes based on a cationic and a helper lipid containing miRNA (LPs/miRNA) were prepared and surface coated with natural or synthetic polymers (NPs/miRNA) and thoroughly characterized physico-chemically. AHCfs were transiently treated with NPs/miRNA vs. LPs/miRNA and DF/miRNA and their viability, miRNA uptake and transfection efficiency were assessed. Hydrogels (HYDs) were prepared through Schiff base reaction of alginate dialdehyde (ADA) [3] and chemically-modified gelatin (GEL-C) and thoroughly characterized. In vitro release kinetics of particles loaded with siRNA-Cy5 embedded into HYD was analyzed. The ability of free and released particles to induce miRcombo-mediated direct reprogramming in vitro was analyzed. Biodistribution was assessed by in vivo trials in infarcted mouse models after intracardiac injection.

RESULTS: NPs/miRNA showed higher miRNA encapsulation efficiency (99% vs. 64%) and biocompatibility (100% vs. 50-60%), and similar cell transfection efficiency compared to commercial DF/miRNA control. LPs/miRcombo and NPs/miRcombo increased in vitro direct reprogramming efficiency respect to DF/miRcombo. Selected HYD showed injectability, cell adhesion ability, biomimetic stiffness, and proper stability. NPs/miRNA showed superior stability and could be delivered through the injectable HYD. Mouse trials showed improved heart biodistribution with NPs-loaded HYD.

DISCUSSION & CONCLUSIONS: LPs/miRNA were efficient and safe but unstable, hence they were suitable for in vitro transfection, only. Novel NPs/miRNA (patented) were efficient, safe and stable when embedded in HYD. Injectable self-healing HYD improved local retention in vivo. NPs/miRNA allowed surface functionalization for cell-targeted delivery.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 772168 (BIORECAR project).

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Keywords: Cardiovascular, Gene therapy

OP-226

Preservation and Vascularization of Cardiac Extracellular Matrix to Promote Cardiac Repair after Acute Myocardial Infarction

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INTRODUCTION: Following myocardial infarction (MI), the upregulated matrix metalloproteinases (MMPs) especially MMP-2/9 lead to the degradation of cardiac extracellular matrix (ECM), leading to impaired cardiac function. Therefore, preservation of cardiac ECM by decreasing MMP-2/9 activity will increase cardiac function. Meanwhile, vascularization is critical for cardiac ECM as otherwise its structure and composition change over time. In this work, a non-toxic peptide CTTHWGFTLC (CTT) that specifically inhibits MMP-2/9 [1], and a pro-angiogenic basic fibroblast growth factor (bFGF) were used to preserve and vascularize the cardiac ECM. A fast gelling, degradable, and reactive oxygen species (ROS)-scavenging hydrogel was developed to deliver CTT and bFGF to the infarcted hearts. We evaluated the efficacy of the delivery system in preserving and vascularizing cardiac ECM, and improving cardiac function.

METHODS: The effects of CTT and bFGF on rat cardiac fibroblast (RCF) differentiation into myofibroblast, and on human umbilical vein endothelial cell (HUVEC) migration and tube formation were determined. TGF β was added to mimic the microenvironment of infarcted myocardium. The thermosensitive, injectable, degradable, and ROS-scavenging hydrogel was synthesized by free radical polymerization of N-isopropylacrylamide, acrylate-polyactide, methacrylate poly(ethylene glycol), and 4-(acryloxymethyl)-phenylboronic acid pinacol ester. CTT and bFGF were encapsulated into the hydrogel by stirring at 40°C. The hydrogel/CTT/bFGF was locally injected into the infarcted tissues at acute myocardial infarction (MI) stage. Cardiac function was monitored by echocardiogram at days 7, 14, 21, and 28. The heart tissue was harvested after 28 days of implantation.

RESULTS: The combination of CTT and bFGF synergistically promoted tube formation of HUVECs compared to the treatment with CTT or bFGF ($p < 0.01$). Interestingly, both CTT and bFGF inhibited myofibroblast differentiation of cardiac fibroblasts. The combination of CTT and bFGF further significantly decreased the differentiation. After 28 days of implantation of the CTT/bFGF/Gel, the wall thickness was significantly increased compared to the CTT/Gel, bFGF/Gel, Gel only and MI. The released CTT and bFGF preserved cardiac ECM, decreased cardiac fibrosis, and stimulated angiogenesis in the infarcted area, leading to a significant increase of cardiac function.

DISCUSSION & CONCLUSIONS: A hydrogel based CTT and bFGF release system was developed to decrease MMP-2/9 bioactivity, preserve cardiac ECM, and vascularize the preserved ECM after MI. The CTT and bFGF showed a synergistic effect in inhibiting myofibroblast formation and promoting angiogenesis.

ACKNOWLEDGEMENTS: This work is supported by National Institutes of Health.

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Keywords: Vascular systems / vascularisation and heart, Drug delivery

OP-227

A superior extracellular matrix binding motif to considerably enhance the regenerative activity and safety of therapeutic proteins

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INTRODUCTION: Therapeutic proteins such as cytokines and growth factors are very important for regenerative medicine. However, these molecules have encountered limited clinical success due to low effectiveness and major safety concerns, highlighting the need to develop better approaches that increase efficacy and safety. Promising approaches to improve their efficacy and safety aim at leveraging how the extracellular matrix (ECM) controls the activity of these molecules during tissue healing.

METHODS: Using a protein motif screening approach, we discovered a novel ECM sequence that possesses an exceptionally strong binding motif for ECM components. We engineered pro-regenerative therapeutics platelet-derived growth factor-BB (PDGF-BB) and interleukin-1 receptor antagonist (IL-1Ra) with this novel sequence to provide an exceptionally high affinity to the ECM. We tested the retention and systemic diffusion of the engineered therapeutics in the tissue when delivered locally. Finally, we tested the regenerative capacity of the engineered PDGF-BB in mouse models of injury, including diabetic skin wound healing and muscle regeneration; whilst also testing engineered IL-1Ra in cardiac repair following myocardial infarction.

RESULTS: When delivered locally in muscle and skin mouse models, the engineered proteins were considerably retained in the tissue and thus had reduced leakage in to the circulation. Importantly, the prolonged retention and minimal systemic diffusion of engineered PDGF-BB abolished the tumour growth-promoting adverse effect that was observed with wild-type PDGF-BB. When delivered locally, engineered PDGF-BB was also substantially more effective at promoting diabetic wound healing and regeneration after volumetric muscle loss, compared to wild-type PDGF-BB. Finally, while local or systemic delivery of wild-type IL-1Ra showed minor effects, intramyocardial delivery of engineered IL-1Ra enhanced cardiac repair after myocardial infarction by limiting cardiomyocyte death and fibrosis.

DISCUSSION & CONCLUSIONS: We demonstrated that engineering therapeutic proteins with an ECM-binding motif confers them with a built-in delivery system that is effective in multiple models of tissue repair and regeneration. This engineering approach highlights the key importance of exploiting interactions between ECM and therapeutic proteins for developing effective and safer regenerative therapies. Finally, this strategy may also be useful beyond regenerative medicine applications where local delivery is necessary to reach therapeutic efficacy at a low dose and limit off-target effects.

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Keywords: Cardiovascular, Biologics and growth factors

OP-228

Functional cardiac organoids modeling the inflammatory environment of myocardial infarction

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INTRODUCTION: Myocardial infarction (MI) is an ischemic and inflammatory event majorly orchestrated by macrophages from infiltrating monocytes (1). These macrophages play a critical role in deciding the fate of the heart post-MI (2). However, there is no cardiac disease model in existence that incorporates an appropriate inflammatory environment. Hence, the aim of this project is to develop a humanised model of MI, using induced pluripotent stem cell (iPSC) derived cardiomyocytes together with inflammatory cytokine stimulation, to model the disease environment.

METHODS: iPSCs were expanded and differentiated to obtain macrophages (iMacs) and cardiomyocytes (iCMs). To understand the effect of iCM secretome on the polarization and maturity of iMacs, iMacs were first cultured in iCM conditioned media, followed by a co-culture of iMacs and iCMs. Phenotypic and functional capabilities of iMacs were assessed using flow cytometry and qRT-PCR. Similarly, the maturity of iCMs in the presence of iMacs was studied using qRT-PCR and immunofluorescence imaging. Next, an electrical stimulation (ES) regime was developed to facilitate iCM maturation. Finally, an engineered heart tissue (EHT) model was developed using type I collagen and Matrigel™ to fabricate 3D models of the myocardium.

RESULTS: The iMacs that were generated were found to be CD14^{high}CD11b^{high} and CX3CR1^{high}HLA-DR^{high}CCR2^{low}. Additionally, iMacs were able to perform phagocytosis as well as polarize to become classically and alternatively activated macrophages. The iCMs generated were cTnThighMLC2a^{high} and performed spontaneous beating from day 7 of differentiation. On observing the effect of iCMs on iMacs, iCM conditioned media was found to drive a pro-regenerative response in iMacs while iMacs in co-culture with iCMs maintained a quiescent phenotype, similar to the control iMacs with no treatment. Finally, the ES regime used was found to support robust maturation of iCMs, with an upregulation in genes such as MY6, MY7, SERCA2 and GJA1.

DISCUSSION & CONCLUSIONS: Firstly, we demonstrate the generation and characterisation of iMacs and iCMs from the same iPSC parent line. Next, we show shown the functional differences in iMacs when cultured with iCM conditioned media as well as in co-culture with iCMs. Finally, we also show successful maturation of iCMs under an optimized ES regime.

ACKNOWLEDGEMENTS: The authors acknowledge SFI for the funding (18/EPSC - CDT/3583) and lifETIME CDT.

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Keywords: Immunity / immunomodulation / macrophage, In vitro microenvironments

OP-229

Engineering Nano Ag-Decorated Graphene Oxide/ Gelatin Methacryloyl Photothermal Platforms to Combat Bacterial Infection and Prompt Wound Healing

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INTRODUCTION: Infected wounds have become a common clinical problem. Due to the abuse of antibiotics, the emergence of drug-resistant bacteria has threatened public health, led to poor curative effects, and even death. Therefore, it is necessary to find new antimicrobial methods to treat drug-resistant bacteria. Nanoparticles are applied widely because of their broad-spectrum antibacterial ability. Ag nanoparticles have become promising antibacterial agents owing to their good biocompatibility, bioactivity, and immunomodulatory effects. However, the therapeutic effects of Ag nanoparticles are generally limited by their easy oxidization and inferior sustained-release effect. As an inorganic nanomaterial, graphene oxide (GO) is rich in functional groups, π - π bonds, and has good photothermal effect that confers itself to combined antimicrobial capacities. Gelatin methacryloyl (GelMA) is a commonly used material to construct hydrogels that has the characteristics of ultraviolet cross-linking, simulated extracellular matrix structure, and sustained release drugs. Consequently, we infer that it's possible to construct an excellent antibacterial material that contains Ag/GO nanoparticles encapsulated into a GelMA hydrogel network.

METHODS: Silver ammonia solution was generated with silver nitrate and ammonia, then reduced to silver nanoparticles by glucose and modified on GO to construct GO/Ag nanoparticles, and finally encapsulated into GelMA hydrogel to obtain Ag-GO-GelMA hydrogel. We evaluated the physicochemical, the rheological properties, photothermal effects, and antibacterial characterization. Then we constructed the rat infected wound model, and the healing effects were evaluated by gross photo and histological staining.

RESULTS: Electron microscopy, FT-IR, XRD, TGA and rheological tests showed that the Ag-GO-GelMA hydrogel was successfully constructed. The fabricated hydrogel exhibited good heating capacity under near-infrared irradiation, accelerated Ag releasing, and excellent antibacterial effects towards *Staphylococcus aureus* and *Escherichia coli*. Cell experiments showed that the Ag-GO-GelMA hydrogel had good biocompatibility with fibroblasts. The Ag-GO-GelMA hydrogel exhibited excellent photothermal, antibacterial, and anti-inflammatory properties in vivo and accelerated wound repair when placed on the infected wound of rats. Histological staining of important organs showed that the Ag-GO-GelMA hydrogel had no obvious biological toxicity.

DISCUSSION & CONCLUSIONS: We have successfully constructed an Ag-GO-GelMA hydrogel, which has excellent mechanical, photothermal, antibacterial, and anti-inflammatory properties. The incorporated GO possessed inherent photothermal effects and could adsorb Ag nanoparticles to reduce oxidation through π - π bonds. The adsorbed Ag nanoparticles could enhance the photothermal effects of GO by reducing nonradiative transition and promoting the dispersion of GO in GelMA hydrogel. Finally, the fabricated Ag-GO-GelMA hydrogel was able to accelerate the healing of infected wounds.

Keywords: Wound healing, Biomaterials

OP-230

Magneto-responsive fibre-based anisotropic hydrogel for spinal cord regeneration

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INTRODUCTION: Spinal cord nerve fibres alignment is crucial for brain-spinal cord electrical communication. The injury-induced loss of neuronal network structure and the restricted regenerative capacity of the central nervous system result in severe impairments of the patient's sensory and motor capabilities. Tissue engineering and regenerative medicine researchers are using biomaterials, cells, and microfabrication to develop spinal cord physiology restoration treatments. We intend to determine if a biomaterial with anisotropic properties that mirror the native tissue's structural complexity is advantageous to one with random fibre orientation.

METHODS: Superparamagnetic Fe₃O₄ nanoparticles were synthesised via co-precipitation and put into a polycaprolactone-gelatine solution. Using an electrospinning system developed by our group [1], highly aligned and random fibrous membranes were manufactured. The aligned fibrous membrane was then microcut into pieces of 100x300 µm. Then, collagen-based hydrogels with aligned fibres were created. A magnetic device consisting of two Helmholtz coils specifically built to hold cell culture plates was used for the alignment. In both instances, the hydrogel was crosslinked at 37 °C, maintaining the placement of the fibres. The viability, proliferation, and differentiation of neural stem cells (NSCs) grown on ordered and disordered substrates with and without Fe₃O₄ were studied.

RESULTS: Electrospinning was used to create fibrous membranes with 80% of the fibres aligned within 15° of the anisotropy axes. According to VSM analysis, Fe₃O₄ imparts superparamagnetic properties to nanofibers. Helmholtz coils generated a 20 mT magnetic field to swiftly position fibrous membranes longitudinally in collagen gel. On anisotropic substrates, NSCs adhered and spread unidirectionally, producing longitudinal interconnections, while on disordered substrates, their morphology was star-shaped. Experiments on differentiation revealed that NSCs can differentiate into neurons and that their neurites grow along the longitudinal axis of the fibres.

DISCUSSION & CONCLUSIONS: We created simple magneto-responsive fibre hydrogels with the intention of using them as minimally invasive injectable hydrogels containing magnetically sensitive fibres that may be oriented in situ toward defined locations. Future studies will incorporate the direct magnetic stimulation of cells in situ. The implantation of this fibrous structure is intended to assist the connecting of neurons between the two damaged stumps of the lesioned spinal cord, thereby restoring the neural network.

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Keywords: Fibre technology, Nervous system (brain-central-peripheral / disorders)

OP-231

Development of an injectable contactless gellan gum-based hydrogel for regenerative medicine

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INTRODUCTION: Cellular alignment is fundamental in human tissue (e.g. spinal cord, muscle, tendon, and cartilage)¹, many techniques have been described to control it, but the resulting 3D-aligned scaffolds usually need invasive surgery to be implanted. Injectable hydrogels can be transplanted via minimally invasive injection, but without displaying any aligned structure. In the present work, we developed an injectable contactless hydrogel based on gellan gum (GG) a biocompatible and biodegradable polysaccharide² and magnetic fibers (MagCs) that can achieve an orientated architecture by applying a static magnetic field (SMF).

METHODS: MagCs are obtained by coupling 1mg/mL iron oxide nanoparticles to collagen type I (1:1, 1:3) at pH 7.4. 1% GG hydrosol mixed with hyaluronic acid (HA, 0-0.6%) and MagCs is injectable through 30G needle at 37°C. The gelation occurs in the presence of cations (e.g. biological fluids) in three minutes. Mechanical properties were investigated by rheometer and dynamic mechanical analysis. Biocompatibility was preliminarily evaluated by embedding or placing in contact murine macrophage (RAW264.7) cell line and macrophages derived from two patients (PrestoBlue assay, live/dead assay, Elisa: IL-10, IL-1 β , IL-4, TNF- α , flow cytometry analysis: CD86, CD14, CD16, HLA-DR). Then the hydrogels were subcutaneously implanted in Male Wistar Han (CrI:WI/Han) rats (12 animals, age=10 weeks). At 1, 3 and 7 days the hydrogels and the surrounding tissue were processed for histological and immunohistochemical analyses. Potential toxicity was evaluated by analyzing the spleen, liver, kidney and lymph nodes.

RESULTS: The hydrogels showed a dependence of stability and mechanical properties (elastic, G' , and viscous, G'' , moduli) on their composition, in particular, the Young's Modulus was 12-19kPa which is comparable to muscle tissue³, stress relaxation behavior was similar to human tissues when HA was added⁴. MagCs embedded into the hydrogels were easily oriented by applying a SMF (400mT) for 3 minutes at a distance of $\approx$ 4cm. Hydrogels exhibited good biocompatibility without affecting cell viability and morphology of RAW264.7 and primary macrophages. No inflammation was detected in rat implants and was observed an aligned structure in vivo in MagCs containing hydrogels.

DISCUSSION & CONCLUSIONS: In vitro analysis pointed out the system biocompatibility that was confirmed during the in vivo test, which also highlighted its ability to achieve an aligned architecture by a remote SMF application during the gelation. In conclusion, we were able to develop a fine-

tunable, contactless system with a well-defined architecture.

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Keywords: Hydrogels and injectable systems, Biomaterials

OP-232

Design of magnetically-assisted cell sheet constructs to study hypoxia and inflammatory cues in tendon inflammation

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INTRODUCTION: Tendon injuries are common and debilitating, aggravated by an impaired healing that frequently fails to restore normal functionality, contributing to the severity of lesion, and increasing the risk for re-ruptures. Although persistent inflammatory cues have been associated to the development of tendon pathologies, the impact of oxygen-sensing pathways in repair mechanisms has not been established despite its relevance in the regulation of inflammatory and fibrotic pathways. Recent studies have found that pulsed electromagnetic field (PEMF) can reduce inflammatory cues on human tendon cells (hTDCs)[1], and in hypoxia-induced inflammation by decreasing TNF α , IL-6 and IL-8 in neuron-like and microglial cells[2].

METHODS: We investigated the effect of hypoxia (1% and 2% of oxygen tension-OT) using magnetic cell sheet (IL-1 β -magCSs) constructs made of hTDCs primed with pro-inflammatory IL-1 β , and magnetic nanoparticles[3]. IL-1 β -magCSs were exposed to 1h, 4h and 6h of low OT in a hypoxic chamber and the inflammatory profile assessed by qPCR, immunochemistry and ELISA assays. To confirm the role of PEMF (5Hz, 4mT, 50% duty cycle) on hypoxia modulation, IL-1 β -magCSs, previously exposed to OT, were 1h-stimulated with PEMF.

RESULTS: Our results show a significant increase in HIF-1 α and HIF-2 α expression on IL-1 β -magCSs after exposure to 2%-OT in all time points, compared to 1%-OT and to normoxia. The TNF α , IL-6 and IL-8 expression also increased in a 6h exposure to 1%-OT. When IL-1 β -magCSs exposed to hypoxia are PEMF-stimulated, there is a decrease in pro-inflammatory genes and an increase in anti-inflammatory, IL-4 and IL-10 expression compared to unstimulated-magCSs. The release of IFN γ , TNF α and IL-6 was increased after 6h-exposure, regardless of %-OT. In contrast, IL-10 levels tend to rise after PEMF-stimulation at 2%-OT in comparison to unstimulated-magCSs.

We also observed an increment in NF κ B expression on IL-1 β -magCSs exposed to 4h and 6h of 2%-OT suggesting a link between NF κ B and the production of pro-inflammatory factors. Moreover, PEMF stimulation showed a significantly decrease of the NF κ B levels on IL-1 β -magCSs.

DISCUSSION & CONCLUSIONS: Overall, low OT enhances expression of hypoxia-associated genes and inflammatory markers in IL-1 β -magCSs with the involvement of NF κ B. PEMF modulates the response of magCSs, previously conditioned to hypoxia and to inflammatory triggers, favouring expression of anti-inflammatory genes and proteins, supporting PEMF impact in pro-regenerative tendon strategies.

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Keywords: Stem cells - general, In vitro microenvironments

OP-233

A multi-responsive chitosan-based self-crosslinked hydrogel for controlled drug delivery

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INTRODUCTION: Controlled drug delivery systems have gradually emerged as a viable alternative to overcome the shortcomings of traditional drug formulations, such as low bioavailability and rapid drop in plasma concentration. Hydrogels are widely studied as drug carriers due to their tunable drug release performance, controllable degradation, low side effects, and ability to protect therapeutic components in hostile physiological environments[1]. In particular, environmentally sensitive hydrogels respond to environmental stimuli (e.g. light, temperature, pH), and thus have a great potential to achieve controlled drug release [2]. In this work, a multi-responsive chitosan-based self-crosslinked hydrogel as a controlled drug delivery system was developed.

METHODS: Aldehyde chitosan (CTS-CHO) with tunable functional group densities was synthesised from chitosan via oxidation reaction. The self-crosslinked hydrogel was fabricated from CTS-CHO via the dynamic Schiff's base reaction. The mechanical properties, multi-responsive properties and thixotropic features, swelling ratios, and degradation profiles of the hydrogel were evaluated. The controlled drug release profiles of the hydrogel were assessed using drug models, BSA and Fluorescein isothiocyanate-dextran (FITC-dextran), in pH 5.7 and 7.4 phosphate buffered saline (PBS) respectively. The cytotoxicity of the hydrogel extract was observed from Live/dead and AlamarBlue assay against HEK 293 cells.

RESULTS: The hydrogels exhibited excellent pH-responsive, thermosensitive, injectable and degradable properties. The release rates of BSA and FITC-dextran from the hydrogel were significantly accelerated when the pH of the PBS was reduced from 7.4 to 5.7 due to the pH-responsive property. The cell viability of CTS-CHO self-crosslinked hydrogel at various experimental concentrations was above 80%. All the results demonstrated that the multi-responsive, CTS-CHO self-crosslinked hydrogel system is a promising candidate for controlled release in various environments, especially for protein and macromolecule drugs.

DISCUSSION & CONCLUSIONS: In this study, a chitosan-based self-crosslinked hydrogel with multi-responsive properties and crucial properties for drug delivery, such as degradability, injectability, self-healable property, and biocompatibility was reported. The hydrogel showed great potential as a drug delivery carrier which can control the release of protein and polysaccharide drugs under two principles, dynamic covalent bond hydrolysis and diffusion, respectively.

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Keywords: Drug delivery, Polymers - natural / synthetic / responsive

OP-234

Autologous epithelial cell sheet with dedicated device for intractable esophageal stricture

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INTRODUCTION: Esophageal stricture remains one of the major unmet medical needs associated with large endoscopic submucosal dissections (ESD) for superficial esophageal neoplasms, or congenital esophageal atresia postoperative anastomotic stricture.

METHODS: In our previous safety and feasibility study, tissue-engineered epithelial cell sheets, produced by culturing the patients' own oral mucosal epithelial cells on temperature responsive culture dishes, were transplanted onto the ulcer surface following ESD in 20 adult patients.

RESULTS: The result of the study indicate that autologous epithelial cell sheets were reproducibly produced, transplanted safely, and promoted early re-epithelialization. Therefore, we started clinical trial for adult patients such as esophageal neoplasms, as soon as conducted clinical research on regenerative therapy using autologous epithelial cell sheets for children with congenital esophageal atresia. This regenerative therapy using epithelial cell sheets has been conducted for three children with congenital esophageal atresia postoperative anastomotic stricture. Safety was confirmed in all cases, and clear efficacy was confirmed in two cases. Based on the results of preclinical studies using a large animal (mini-pig) esophageal stricture model, we believe that the therapeutic concept of epithelial cell sheet application to the site of mucosal defect after balloon dilation reduces local fibrosis and restenosis.

DISCUSSION & CONCLUSIONS: While continuing to promote this human clinical research, develop manufacturing and quality control of pediatric sheets, and develop pediatric sheet implantation devices. We will optimize the size and shape of the pediatric sheet implantation device while conducting clinical research. The field of pediatric refractory stricture for postoperative congenital esophageal atresia is an unmet medical need, but both number of cases and the market size is small, making it difficult for companies to invest in development. Therefore, academia will promote the development of pediatric regenerative medicine products and out-license the technology to companies to develop new regenerative therapies that can become a global standard treatment for intractable pediatric diseases.

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Keywords: Cell therapy, Translation and commercialisation (inc. clinical trials and regulatory approval)

OP-235

The Use of Self-Assembling Peptide Hydrogels to Deliver Cellular Therapies for Peripheral Nerve Reconstruction

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INTRODUCTION: Current engineered nerve guidance conduits (NGC) are only suitable for small peripheral nerve gaps. Larger defects require nerve grafting, associated with donor site morbidity and poor outcomes. Failure of current NGC to support regeneration across larger defects are due to a lack of cellular/glial support to the regenerating nerve. To improve upon current NGC, some form of cellular therapy is likely required, but this is hindered in translation e.g. due to scalability, safety, regulation and cost. The stromal vascular fraction (SVF), derived from adipose tissue, is a stem cell rich tissue isolate that can be administered and isolated in a single procedure (therefore easily translated). Self-assembling peptide hydrogels (SAP) are synthetically produced, biologically safe, injectable hydrogels whose mechanical properties are easily tuned. We optimised our NGC (POLYNERVE®) by adding an SAP hydrogel seeded with SVF and present our findings investigating the delivery of SVF in SAP and the resultant effect on neural regeneration in vitro and in vivo.

METHODS: In vitro, we assessed the long-term viability of SVF in 3D culture within SAP hydrogels of varying stiffness (G' 1–10kPa). In the same SAP hydrogels, we also assayed 3D neuronal outgrowth (from rat dorsal root ganglion neurons) using confocal microscopy. In vivo, utilising a rat sciatic nerve defect model (n=6), we reconstructed a 1cm defect with conduits containing either a) SAP b) SAP + SVF c) collagen or d) autograft. We assessed neuronal regeneration and functional recovery at 6 & 12 weeks with histology (immunostaining), assessment of myelination (transmission electron microscopy), gastrocnemius/soleus weights, functional motor recovery (maximum isometric force testing) and sensory recovery (Hargreaves and Von Frey tests).

RESULTS: In vitro, SAP hydrogels with G' 1-2.5kPa enabled long term SVF viability and proliferation, this finding was mirrored in 3D DRG neuronal outgrowth which was also evident only in softer hydrogels. In vivo, we demonstrated that our novel composite device enabled equivalent histological, motor & sensory recovery to autograft including remyelination. Motor recovery was significantly (n=6) greater in the SAP + SVF group at 12 weeks than those without SVF.

DISCUSSION & CONCLUSIONS: SAP support long-term SVF survival and provide an effective method of transplanting this population within an NGC. In a rodent model, their performance supporting neuronal regeneration appears equivalent to autograft. Our future work will focus on tracking the fate of transplanted SVF in vivo and assess our composite conduit in a large animal model.

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Keywords: Biomaterials, Stem cells - general

OP-236

Development and First Use of a Biological Woven Vascular Graft as an Arteriovenous Shunt in Sheep

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INTRODUCTION: Vascular access is the lifeline of a patient on hemodialysis. Current options have limited durability and are prone to thrombosis, intimal hyperplasia and infections. To overcome these limitations, biological Tissue-Engineered Vascular Grafts (TEVGs) with tunable structural and mechanical properties were developed. The approach relies on the use of Cell-Assembled extracellular Matrix (CAM) yarn that are woven into a biotextile vascular graft. This study aims to demonstrate the development of a woven TEVG with suitable structural and mechanical properties for implantation, and its first use as an arteriovenous shunt (AVS) in a large animal model.

METHODS: CAM sheets were produced by ovine fibroblasts in culture. TEVGs were woven with two sets of CAM yarn, the warp (longitudinal yarns) and the weft (circumferential yarn). CAM sheets were either cut into 5-mm-wide ribbons, which were used as warp, or cut following a spiral pattern to obtain two 1.5-m-long ribbons, which were twisted together (5 rev/cm) and used as weft. TEVGs were produced using several combinations of weaving parameters and further evaluated in vitro. TEVGs with the best design were sterilized (Ethylene Oxide) and implanted in sheep as an end-to-side 4-cm-long AVS between the carotid artery and the jugular vein for up to 8 weeks (n=4, diameter: 6 mm). Synthetic grafts (ePTFE) were implanted contralaterally as controls. Doppler echography was realized immediately post-implantation and every two weeks until sacrifice to assess graft patency, lumen diameter and blood flow.

RESULTS: Optimized woven TEVGs had lower water permeability than the 50 mL/min/cm² often suggested for clinical use (25 ± 8 mL/min/cm²), excellent suture retention strength and burst pressure (5 ± 1 N and 3492 ± 307 mmHg, respectively), and higher compliance than ePTFE prostheses (4.8 %/100 mmHg vs 1.2 %/100 mmHg). Surgical implantation of TEVGs as AVS was successful and no blood leakage was observed at the anastomosis or through the wall after blood flow was restored. In addition, graft remained patent with no signs of thrombosis, stenosis or inflammatory reaction for up to 8 weeks. Grafts were successfully cannulated at 4 weeks and hemostasis was rapidly achieved after removal of the needle.

DISCUSSION & CONCLUSIONS: We showed that a biological, allogeneic, woven TEVG with structural and mechanical properties closer to those of native blood vessels can be produced and used as a vascular access conduit in a large animal model.

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Keywords: In vivo and animal models, Biofabrication

OP-237

Biomaterial-specific regulation of early vascular graft healing

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INTRODUCTION: Cardiovascular disease (CVD) remains the most common cause of death worldwide. Advanced CVD often requires the substitution of diseased vessels. Autologous vessels, such as the saphenous vein or internal mammary artery, still remain the gold standard for vascular grafting. Synthetic conduits perform well for large-diameter applications but fail to serve as small-diameter vascular grafts (SDVGs). Detailed knowledge about the processes during graft healing could help to improve synthetic materials to fabricate a long-term patent SDVG. The current study aims to elucidate the molecular mechanisms involved in the early healing phase of different SDVG materials.

METHODS: Autologous aorta, expanded polytetrafluorethylene (ePTFE) and biodegradable thermoplastic polyether urethane-urea (TPUU) conduits were implanted as infrarenal aorta substitutes in rats. Grafts were retrieved after 24 h and 7 days and analysed with cytokine arrays and gene expression screening of the perivascular adipose tissue (PVAT) was performed. Furthermore, extra-cellular vesicles from the PVAT were subjected to miRNA analyses.

RESULTS: Several pro-inflammatory and atherosclerosis-associated cytokines were upregulated in the anastomotic regions of ePTFE implants after 24 h. After 7 days, ePTFE conduits showed a significantly higher expression of IGF-1 growth factor. Moreover, significant differences in resident adaptive immune cell populations between PVATs of ePTFE and TPUU implants were found. The expression of remodeling and inflammatory markers in the PVAT in all implants was partly mediated by extra-cellular vesicle bound miRNAs.

DISCUSSION & CONCLUSIONS: The higher expression of distinct cytokines in the ePTFE group indicated that ePTFE grafts are more susceptible to intimal hyperplasia and atherosclerotic plaque formation than autologous implants or TPUU grafts. Furthermore, resident immune cell populations in the PVAT may contribute to remodeling and healing of the grafts from the adventitial side by extra-cellular vesicle mediated miRNA interactions.

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Keywords: Biomaterials, In vivo and animal models

OP-238

Development of a biomimetic multi-layered functionalised antimicrobial scaffold for advanced wound healing

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INTRODUCTION: Diabetic foot ulcers (DFUs) are chronic non-healing wounds that constitute one of the most devastating complications of diabetes. Dysfunctional angiogenesis plays a key role in DFU, limiting tissue repair leaving wounds open and prone to infection¹. Collagen-glycosaminoglycan (CG) scaffolds have shown promise to promote burn wound healing², but further functionalisation is needed for these complex wounds. The aim of this project is to develop a bi-layered scaffold with an epidermal antimicrobial collagen/chitosan film to prevent wound infection and a pro-angiogenic functionalised dermal porous CG scaffold layer to heal complex wounds.

METHODS: Antimicrobial collagen/chitosan films (0.5%, 1% / 0.75%) were combined via lyophilisation with a type I collagen, chondroitin-6-sulfate slurry and mechanical properties were optimised through film composition and crosslinking treatment. The collagen/condroitin-6-sulfate slurry was then functionalised with either fibronectin, collagen IV, or laminin-111 to enhance its angiogenic potential.

The film's antimicrobial potential was tested versus staphylococcus aureus. The vascularisation potential of the scaffold was investigated through the culture of human umbilical vein endothelial cells (HUVECs), and human dermal fibroblasts (HDFs) on the functionalised CG scaffold layer.

RESULTS: The bi-layered scaffolds were fabricated with robust good mechanical properties for implantation (compressive modulus = 1.83 ± 0.0035 kPa, adhesion strength between layers = 1.6 ± 0.38 N). Biological characterisation showed that all collagen/chitosan films had antimicrobial activity versus staphylococcus aureus regardless of crosslinking regime and composition, but crosslinked scaffolds showed enhanced infiltration and growth of vascular cells. In comparison to other molecules, laminin-111 showed a significant increase in vascular endothelial growth factor (VEGF) production from HDFs over 7 days (1044 ± 112 pg/ml) when incorporated into the scaffold and HUVECs seeded on these scaffolds formed tubular structures.

DISCUSSION & CONCLUSIONS: The collagen/chitosan film layer demonstrated the ability to inhibit growth and infiltration of staphylococcus aureus, the most common bacterial isolate found in infected wounds³. Functionalisation of the CG scaffold layer with laminin-111 enhanced the angiogenic potential of the dermal scaffold layer, with HDFs producing significantly more pro-angiogenic cytokines (VEGF) and supporting tubular structure formation by HUVECs within the scaffold, demonstrating the scaffold's pro-angiogenic potential. Taken together, these results demonstrate the promise of this bi-layered scaffold as a biomaterial-based solution to treat DFUs.

ACKNOWLEDGEMENTS: Science Foundation Ireland (SFI), AMBER Centre, Grant No.: SFI/12/RC/2278_2

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Keywords: Biomaterials, Wound healing

OP-239

An implantable microscope objective-on-a-chip for multiphoton imaging in vivo

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INTRODUCTION: Regulations for biomaterial testing rely on histopathological analyses of animal biopsies leading to ethical issues(1). In this context, intravital microscopy techniques allow to quantify in vivo the foreign body reaction to the implant reducing the number of animals required to statistically validate the biomaterial(2,3). However, traditional windows chambers are invasive, lead to strong inflammatory reactions and fail in the reliability of long-term evaluations.

METHODS: Therefore, we developed a system of microlenses, coupled to a microsc scaffold, both incorporated in a miniaturised imaging window(4). The chip is microfabricated by two-photon polymerization (2PP) of a biocompatible photoresist called SZ2080(5). The chip is designed to act as an in-situ microscope objective with the aim to overcome the restrictions of in vivo imaging related to tissue-induced spherical aberrations. The chip includes fluorescent beacons, allowing optical alignment for multiple observations. Firstly, we established the microlenses process of fabrication as 2PP of the outer shell followed by the UV bulk polymerization of SZ2080(6). Then, we developed a dedicated protocol to fabricate both the microlenses and the 3D microsc scaffolds on the same chip, and we obtained a prototype of the integrated smart microstructured imaging window.

RESULTS: Once we proved the reliability of the microfabrication process, we independently validated the microlenses morphological and optical quality. Then, we quantified their dioptric power and magnification by coupling them to low numerical aperture objectives, to image stained cells cultured in vitro both on flat substrates and inside the 3D microsc scaffolds. Remarkably, the fabricated microlenses allow to efficiently excite the fluorescence of labelled cells and collect high resolution, magnified images of them on a conventional two-photon scanning microscope.

DISCUSSION & CONCLUSIONS: The chip enhanced the possibility to reduce optical aberrations related to intravital imaging. Thanks to these encouraging results, the chip will now be used for in vivo observations of the host inflammatory response to the implant of biomaterials, in the aim (a) to reduce the number of animals sacrificed in biomaterial testing procedures and (b) strengthening the validation protocols in both a qualitative and quantitative manner.

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Keywords: Imaging - advanced, Imaging - in vivo

OP-240

Volumetric bioprinting for spatial patterning of biomolecules and biofunctionalization of thiol-ene, gelatin-based constructs in tomographic biofabrication

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INTRODUCTION: Three-dimensional (3D) bioprinting technologies are powerful tools for biomedical research, given their ability to shape cell-laden biomaterials into customized architectures. With the recently developed light-based, tomography-inspired technique called volumetric bioprinting (VBP), large cellularized constructs can be printed in tens of seconds at high resolutions[1]. Herein, we introduce a new strategy to biofunctionalize VBP constructs with precise spatio-temporal resolution across centimeter-scale hydrogels. To achieve this, we developed hydrogel biomaterials based on norbornene-modified gelatin (gelNOR) that are mechanically tunable and enable spatially precise 4D-photografting in response to tomographic light exposure in a volumetric printer.

METHODS: GelNOR (80%DOF) was synthesized and crosslinked with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP; 0.1w/v%) and different thiolated crosslinkers (DTT and ethyleneglycol dithiol). The mechanical and photorheological behavior of the bioresins was evaluated, and their VBP printing resolution and accuracy was assessed. 4D-photografting of fluorescently tagged, thiolated compounds and proteins onto complex VBP constructs post-printing using the volumetric printer was first shown with geometrical 2D masks, and later using complex 3D structures. Grafting accuracy was assessed through fluorescence intensity quantification and 3D fluorescence imaging.

RESULTS: Mechanical and photorheological characterization of gelNOR hydrogels showed that modifying the length of the thiolated crosslinker (DTT= $\sim 5.4\text{\AA}$ or ethyleneglycol dithiol= $\sim 11.3\text{\AA}$) yielded a wide range of stiffnesses ($\sim 1\text{kPa}$ - 20kPa) and tunable crosslinking kinetics. These bioresins could be volumetrically printed, achieving high resolutions of positive ($23.7\pm 10.8\mu\text{m}$) and negative ($176.8\pm 36.3\mu\text{m}$) features. Convolutd structures (e.g. gyroid, hollow torus knot) were printed with high accuracy. Photografting of thiolated fluorescent compounds in a spatially controlled manner was achieved through an extensive optimization of light dose, initiator concentration and additives modulating the bioresin's reaction kinetics. This optimization allowed for the precise grafting of complex structures within architecturally complex VBP-prints.

DISCUSSION & CONCLUSIONS: We demonstrate the physicochemical versatility of gelNOR hydrogels. Given their high printing accuracy, this family of bioresins shows promise for the creation of highly complex biological structures that mimic the microstructural and mechanical properties of native tissues. They also enable the spatial patterning of molecules of interest (e.g. growth factors and chemoattractants), currently being studied to zonally modulate angiogenesis and vascular infiltration in biofabricated tissues. The ability to chemically edit printable hydrogels through the spatially controlled, covalent functionalization of thiolated compounds (moieties abundantly present in native proteins) poses great potential to dynamically enhance the biological performance of bioprinted

constructs.

ACKNOWLEDGEMENTS: European Research Council (ERC)-European Union's Horizon 2020 research and innovation program (grant agreement No. 949806, VOLUME-BIO)

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Keywords: Biomaterials, Biofabrication

OP-241

Skin biofabrication using tissue units and 3D-bioprinting

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INTRODUCTION: Skin transplantation for treatment of extensive burns is limited by donor site availability. *In vitro* culture and expansion of autologous cells require specialized facilities, is time-consuming, and fail to recapitulate the full diversity of cell phenotypes and extracellular matrix components. By employing micron sized skin grafts (micrografts), or alternatively cell laden porous gelatin microcarriers (PGM), in combination with 3D-bioprinting, the present study aimed at developing less invasive methods for generating tissue engineered skin for transplantation.

METHODS: Skin was obtained from healthy patients undergoing plastic surgery (Swedish Ethical Review Authority approval 2018/97-31). Micrografts were harvested using a novel microdrill technology (US patent 10,932,813) and cultured *in vitro* for up to two weeks. Carboxyfluorescein succinimidyl ester (CFSE) was used to stain micrografts, and bioprinting enabled generation of constructs that were transplanted to an *ex vivo* wound model. PGM were functionalized with (1R,8S,9S)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate (BCN-NHS). CFSE-stained fibroblasts were seeded on the PGM-BCN-NHS for three days to ensure proper attachment and expansion on the microcarriers. Live-dead assay using Calcein AM and Propidium Iodide (PI) was conducted to confirm viability of seeded cells. Constructs were created by adding hyaluronic acid (HA) functionalized with bicyclo[6.1.0]nonyne (BCN) and crosslinked with poly(ethylene glycol) azide (PEG azide) before bioprinting. Constructs were then transplanted to an *ex vivo* wound model and cell migration and proliferation was followed.

RESULTS: Micrografts ranging from 0.5-1 mm were obtained using the microdrill. Explant cultures resulted in a diversity of cells with maintained phenotypes and biochemical properties. Bioprinted micrograft constructs were successfully transplanted to *ex vivo* wounds and migrating cells could be tracked using CFSE. Functionalized PGM enabled bioprinting to form skin constructs, while maintaining high cell viability. Following transplantation of constructs to *ex vivo* wounds, cells were observed to migrate and proliferate within the tissue, thus contributing to tissue recapitulation.

DISCUSSION & CONCLUSIONS: The use of micrografts minimizes needed donor sites for autologous transplantation, while significantly reducing *in vitro* culture time before transplantation. Future studies will evaluate effects on tissue regeneration following skin tissue units in combination with 3D-bioprinting.

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Keywords: 3D printing and bioprinting, Biomaterials

OP-242

Visible light-mediated photo-crosslinking of sugar beet pectin for 3D bioprinting

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INTRODUCTION: Visible light-mediated crosslinking is an attractive method for fabricating three-dimensional (3D) constructs in biomedical applications. In particular, sodium persulfate (SPS) and ruthenium II trisbipyridyl chloride ($[\text{Ru}(\text{bpy})_3]^{2+}$) has been widely used to induce hydrogelation of polymer derivatives possessing phenolic group (Polymer-Ph). In this study, we explored the possibility of using sugar beet pectin (SBP) to fabricate 3D constructs. SBP naturally possesses phenol in its feruloyl group,[1] which eliminates the necessity of conjugating phenolic groups such as those in Gelatin-Ph and Hyaluronic Acid-Ph.[2] Herein, we investigated the hydrogelation of SBP obtained via photo-crosslinking and its application in 3D bioprinting.

METHODS: 3D constructs were fabricated by irradiating visible light (intensity at 405 nm: 150 W/m^2) on bioink consisting of SBP, SPS, $[\text{Ru}(\text{bpy})_3]^{2+}$, and hepatocellular carcinoma (HepG2) cell line, using an extrusion printing system. Hydrogelation kinetics and mechanical properties of the SBP hydrogels were evaluated. Additionally, analysis of the printability and cell viability in the cell-laden constructs was conducted.

RESULTS: SBP hydrogels were successfully fabricated via visible light-mediated crosslinking. The gelation time and mechanical properties of the hydrogels can be controlled by tuning the light intensity, irradiation time, as well as concentration of SPS and $[\text{Ru}(\text{bpy})_3]^{2+}$. 3D constructs with high fidelity were obtained from the SBP inks, which with a suitable condition could form hydrogel within 4 s of irradiation. Encapsulated HepG2 cells showed high viability ($> 85\%$) for 7 days in the cell-laden constructs. In addition, HepG2 cells spontaneously formed spheroids in the 3D constructs.

DISCUSSION & CONCLUSIONS: Based on these results, visible light-mediated crosslinking allows the fabrication of SBP hydrogels with tuneable hydrogelation kinetics and mechanical properties, with minimum effect on cell viability. Taken together, our result demonstrates the great potential of visible light-mediated hydrogelation of SBP and 3D bioprinting using the hydrogelation system for tissue engineering and biomedical application.

ACKNOWLEDGEMENTS: W.M. is supported by JSPS Postdoctoral Fellowship in Japan.

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Keywords: 3D printing and bioprinting, Biomaterials

OP-243

Developing Biodegradable Polymer Resins for Additive Manufacturing via a Novel Synthesis Method

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INTRODUCTION: Photocurable degradable materials are increasingly popular for building 3D scaffolds for tissue engineering via vat photopolymerisation and 2 photon polymerisation (2PP). Our focus is to develop photo-curable multi-arm polycaprolactone (PCL), where the PCL is rendered photocurable via functionalisation by (meth)acrylation. Methacrylation is popular, because of a straightforward and clean synthesis route with methacrylic anhydride, but produces prepolymer resins that are less reactive than acrylates. For example, PCL methacrylate has shown not to be suitable for 2PP, which provides ultra-high resolution sub-micrometre structures.

Acrylates exhibit higher reactivity but their conventional synthesis produces organic amine salts which leads to difficulty on purifying and risk of cytotoxicity.[1] Here we present a novel method to synthesise PCL acrylate via Steglich Esterification, to avoid this risk but still with a high functionality and we show that PCL acrylate shows excellent potential for 2PP.

METHODS: We synthesised our 4-arm PCL via ring-opening reaction between pentaerythritol and ϵ -caprolactone, then functionalised it in dichloromethane solution by using acrylic acid with carbodiimide and 4-dimethylaminopyridine.[2] The chemical structure of 4-arm PCL acrylate (4PCL_A) was characterised by nuclear magnetic resonance (NMR) spectroscopy. We fabricated PCL (meth)acrylate film samples for cell culture to test the viability (via resazurin assay) and dog-bone samples for tensile testing. Tubes and microscale 3D structures were fabricated via UV-based and femto-second 2PP laser additive manufacturing respectively, both of which were characterised by Scanning Electron Microscopy (SEM).

RESULTS: NMR results show that the acrylate groups have been successfully introduced to 4-arm PCL. The degree of acrylation is approximately 85%. The mechanical testing results show that the Young's Modulus, ultimate tensile strength, and the max elongation of the bulk material of 4PCL_A are 1.7 MPa, 120 kPa, and 30%, respectively. 3D microscale structures can be observed under SEM, of which the resolution is approximately 1 μ m. Thin films have been assessed by neuronal cell growth and total cell metabolic activity as measured by resazurin is higher compared to 4-arm PCL methacrylate films.

DISCUSSION & CONCLUSIONS: Efficient acrylation functionalisation on PCL can be achieved via Steglich Esterification. 4PCL_A is suitable for both UV-based and 2PP laser additive manufacturing to fabricate ultrahigh-resolution structures, which can be potentially applied for soft tissue engineering. A higher amount of neuronal cell growth has been achieved on PCL-A compared to PCL-MA which indicates excellent neuronal compatibility.

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Keywords: Polymers - natural / synthetic / responsive, Additive manufacturing

OP-244

Low-intensity pulsed ultrasound stimulation modulates neurotrophic factor secretion and inflammation in Schwann cells

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INTRODUCTION: Low-intensity pulsed ultrasound (LIPUS) is a particular stimulation regime that can be used to wirelessly transmit mechanical energy to cells and tissues, inducing beneficial effects in a non-invasive way [1]. In the context of peripheral neuropathies (PNP), LIPUS demonstrated a certain potential in regeneration and inflammation modulation [2], [3]; however, the results are often not repeatable and the optimal ultrasound protocol to maximize effectiveness is unknown, at the moment. In this work, we optimized the LIPUS treatment, looking for best frequency and intensity able to induce neurotrophic factors secretion in a hydrogen peroxide (H₂O₂)-treated Schwann cell line (RSC96), chosen as an appropriate model of PNP. Then, the best conditions found were tested in a human pro-monocytic cell line (THP-1) to evaluate alterations in pro-inflammatory cytokine release.

METHODS: RSC96 cells were provided with H₂O₂-supplemented medium to mimic an oxidative stress injury. Then, LIPUS stimuli [4] were provided for 5 min/day, for 3 days, using different combinations of frequencies (38 kHz, 1 MHz, 5 MHz) and intensities (20, 100, and 500 mW/cm²) and evaluating neurotrophic factor release (β -NGF, BDNF, and GDNF) with ELISA assays. The optimal combination was then tested on THP-1 cells, first differentiated into adherent macrophages with 100 ng/mL PMA for 24 h and then induced to an inflammatory condition by treating them with 1 μ g/mL LPS, evaluating the release of TNF- α , IL-1 β , IL-6, IL-8, and IL-10 with ELISA assays.

RESULTS: 5 MHz and 500 mW/cm² showed the highest efficacy in boosting the production of β -NGF, BDNF, and GDNF. Moreover, ROS level and TNF- α production were significantly reduced. The release of TNF- α , IL-1 β , IL-6, IL-8, and IL-10 was evaluated in THP-1 macrophages, comparing the effects of LIPUS on inflammatory cytokines release with respect to a non-stimulated control.

DISCUSSION & CONCLUSIONS: This study investigated the bioeffects induced by LIPUS on a PNP in vitro model, also exploring inflammation modulation. Further efforts will investigate bioeffects aiming at nerve regeneration and specific pathways involved in intracellular mechano-transduction. Clinical translation will also be pursued, on PNP patients.

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Keywords: Nervous system (brain-central-peripheral / disorders),

OP-245

Mechanical Stimulation of Bone Cells Enhances the Angiogenic Potential of the Secretome and Secreted Extracellular Vesicles

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INTRODUCTION: Angiogenesis is vital for bone repair, as new vessels allow for the migration of osteoprogenitor cells and growth factors. Bone cells are known to release pro-angiogenic factors that promote the formation of new vessels¹. Mechanical loading has been shown to enhance the regenerative potential of the secretome². However, the effect of mechanical stimulation on the angiogenic potential of the secretome and isolated extracellular vesicles (EVs) is largely unknown. Therefore, the aim of this study is to examine how mechanical stimulation of bone cells affects the regenerative potential of the secretome (and secreted EVs), by analysing its effect on angiogenesis. **METHODS:** MLO-Y4 osteocyte-like cells, human osteoblasts (hOBs) or hMSCs were subjected to oscillating fluid shear of 1Pa, 1Hz, using a custom-made parallel plate flow bioreactor for 2h. The cells were cultured for further 24h and the conditioned media (CM) was collected. EVs were isolated through ultracentrifugation. HUVECs were seeded on Geltrex-coated plates to assess tube formation. CM from the three cell types or EVs derived from MLO-Y4s (1µg/ml) were added to the cells at a 1:1 ratio with HUVEC growth media. 10 ng/ml of VEGF was used as a positive control. Following 18h in culture, angiogenesis was quantified in terms of number of junctions, segments, and total tube length.

RESULTS: Following supplementation with osteocyte, osteoblast, or MSC CM, the static groups presented similar tube formation to that of the negative control. This is confirmed upon quantification, where these two groups have comparable numbers of junctions and segments. More complex tubule structures can be seen in the flow groups, as opposed to the static and negative control groups. This is confirmed by a trend towards an increase between the flow group and the negative control in both number of junctions (1.4-fold, 1.6-fold and 1.4-fold) and number of segments (1.6-fold, 1.7-fold and 1.6-fold). Interestingly, following supplementation with EVs derived from MLO-Y4 CM, a significant increase in the number of junctions with a trend of increased number of segments in tube formation was found in the EVs derived from the flow groups when compared to that from the static group.

DISCUSSION & CONCLUSIONS: These results suggest that mechanically stimulated bone cells, independent of stage of lineage commitment, produce an angiogenic secretome. Therefore, these findings highlight the therapeutic potential of secreted factors such as EVs that can be harnessed for the treatment of bone defects.

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Keywords: Bone and bone disorders (osteoporosis etc), Extracellular vesicles

OP-246

3D Printed Gyroid Scaffolds to Control Mechanical Activation of Latent TGF- β 1 under Compression and Shear

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INTRODUCTION: Articular cartilage defects are a challenging issue, with currently no resolute solution. In vitro models are powerful tools to study tissue repair processes, expediting the development of new therapies. Transforming growth factor beta-1 (TGF- β 1) is among the most studied growth factors in chondrogenic differentiation of human Mesenchymal Stromal Cells (hMSCs). TGF- β 1 is secreted by cells in an inactive latent form and can be activated by multiple mechanisms, including mechanically. Previous studies reported the mechanical activation of latent TGF- β 1 under loading.^{1,2} Additionally, 3D printing is an established technique to engineer scaffolds for in vitro models.³ The aim of this study is to investigate the mechanical activation of latent TGF- β 1 using 3D printed scaffolds of various stiffness under controlled compression and shear loading using a custom-made bioreactor.

METHODS: *Scaffold manufacturing:* Scaffolds ($\varnothing$ 8mm, 4mm height) were punched out from an Ultimaker 3 printed sheet of thermoplastic polyurethane (four groups: 15%, 16.9%, 19%, 25% infill densities with gyroid infill). *Compression tests:* Instron 5866. *Bioreactor tests:* All groups, impregnated with fibrin and covered in cell culture medium containing 50 ng/mL latent TGF- β 1 (R&D Systems). *Loading protocol:* 20% compression, 2 Hz shear, 6h. *Active TGF- β 1 detection in medium:* ELISA (DuoSet DY240, R&D Systems).

RESULTS: Scaffolds showed increasing stiffness with increasing infill densities, from 0.1 MPa (15%) to 1 MPa (25%). The concentration of active TGF- β 1 increased 6, 5, 8 or 4-fold for loaded groups with 15%, 16.9%, 19%, or 25% infill densities, respectively, versus their free-swelling controls. Therefore, the activation of latent TGF- β 1 appears stiffness dependent with the highest activation for Young's Modulus around 0.8 MPa.

DISCUSSION & CONCLUSIONS: Gyroid scaffolds were proven to be suitable for mechanical activation of exogenous latent TGF- β 1, using a loading protocol that resembles the natural walking frequency. In the future, mechanical activation of latent TGF- β 1 could be tested using different loading protocols, exploiting the compression-dependent scaffolds' behaviour. This highlights the possible clinical relevance of the model to establish loading protocols that enhance TGF- β 1 activation, which could lead to enhanced regeneration processes in-vivo. Next, we will investigate the activation of endogenous latent TGF- β 1 produced by encapsulated hMSCs. This will provide important information that could be applied to rehabilitation protocols after cell therapy, aiming for an endogenous regeneration process.

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Keywords: Biologics and growth factors, Bioreactors

OP-247

Piezoelectric biomaterials for neural tissue engineering applications

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INTRODUCTION: Due to their electrical activity, nervous system responds naturally to electrical signals. Tissue engineering research relies on the fact that when a critical injury takes place, it is essential to reestablish and to mimic the specific tissue microenvironment. Taking advantage of electroactive piezoelectric materials for scaffolds development, electrical signals can be locally induced making them a promising approach for neural repair. The polarization state of a material and the consequent variations in surface charge allows to influence cellular adhesion, proliferation and differentiation [1]. Surface charged piezoelectric poly(vinylidene fluoride) (PVDF) has proven to enhance material biofunctionality and has already been successfully used for bone and muscle TE [2]. This work reports on the effect of surface charged PVDF films (positive, negative, and non-poled (average neutral surface charge) on adhesion, proliferation and differentiation of neural-derived cell line, under static and dynamic mechano-electrical stimulation.

METHODS: The influence of piezoelectric PVDF with different surface charges and different dynamic mechano-electrical stimuli on neuron-like cells adhesion, proliferation and differentiation was addressed. Adhesion was assessed through vinculin staining, proliferation with DNA content measurement by CyQUANT, immunofluorescence with DAPI and TRITC staining and differentiation through tubulin staining, morphology visualization and neurite measurement.

RESULTS: It is successfully demonstrated that both surface charge and electrically active dynamic microenvironments allow to improve neuron-like cells adhesion, proliferation, and differentiation.

DISCUSSION & CONCLUSIONS: Upon stimulation, PVDF with average positive surface charge allows to significantly improve cellular adhesion compared to PVDF with negative or without average surface charges. It is more favorable for proliferation if the electrical stimulus is provided after 3 hours of cells being in contact with the PVDF scaffolds (initial attachment), with the piezoelectric dynamic stimulation enhancing the surface charge effect. Independently of the PVDF surface charge type, the applied electrical stimulus throughout the assay improved neurite extension and differentiation. These findings provide new knowledge to develop effective approaches for preclinical neural applications.

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Keywords: Electroactive materials, In vitro microenvironments

OP-248

Tissue engineering based approaches relying on mechano-electrically active microenvironments for novel cancer therapies strategies

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INTRODUCTION: Cancer is one of the most malignant diseases and one of the leading cause of death worldwide [1]. Between the different types of cancers, sarcomas identifying cancers that develop from connective tissue anywhere on the body (e.g. muscles, fat, and bones) can lead to aggressive solid tumors [2]. In this case, their incidence is higher during childhood and adolescence. The current therapeutic strategies include conventional chemotherapy, radiation therapy and surgical resection. However, current investigations search for less aggressive and effective approaches once prevention approaches are more acceptable and outweigh the potential risks. In this sense, a promising strategy is the development of therapies based on tissue engineering (TE) concepts, as these strategies allow the modulation of cell behavior through the implementation of structures that act as support (scaffolds) and the application of biochemical or biophysical stimuli.

In this work we introduce electroactive scaffolds, namely piezoelectric ones, typically used for bone and skeletal muscle regeneration as an approach to address/mitigate sarcoma related cancers.

METHODS: Piezoelectric poly(vinylidene fluoride) (PVDF) films were produced by solvent casting. In order to obtain films with different surface charge the films were submitted to electrical poling. Magnetoelectric films were also produced by introducing magnetic nanoparticles: CoFe₂O₄/PVDF and Fe₃O₄/PVDF. Piezoelectric and magnetoelectric polymers were produced and characterized in order to investigate the influence of the mechano-electrical stimuli and consequently their performance in the development of novel cancer therapy strategy.

For that, a mechanical bioreactor is used for the piezoelectric biomaterials, and a magnetic bioreactor for the magnetoelectric ones; and osteosarcoma and rhabdomyosarcoma (RD) cells were used.

RESULTS: Regarding rhabdomyosarcoma cells, it was verified that the presence of surface charge enhances their proliferation. When the samples were subjected to mechano-electrical stimuli, it was observed that the PVDF films with positive charge promote the decrease of the RD cell proliferation. In relation to osteosarcoma cells, it was verified that the mechano-electrical stimulus also promoted the decrease of the cell proliferation.

Interestingly, the stimuli responsible for the decrease of the sarcoma cells, allow to improve proliferation of healthy cells, allowing regeneration of the damage tissue.

DISCUSSION & CONCLUSIONS: It is successfully demonstrated that electrically active dynamic microenvironments can be suitable to develop new effective cancer therapy strategies, together with a regeneration of the damage tissue.

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Keywords: Cancer, Electroactive materials

OP-249

Platelet-derived Extracellular Vesicles Promote Stem Cells Tenogenic Commitment in a Bioengineered Tendon 3D Model

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INTRODUCTION: Worldwide, tendon disorders are one of the main causes of disability that decrease the quality of life of individuals and represent a substantial economic burden on society. Currently, the main therapies used for tendon injuries are not able to restore tendon functionality, and due to tendons' hypovascular and hypocellular nature, they present a reduced healing capacity, which also limits the success of the available therapies (1). In order to discover new therapies, extracellular vesicles (EVs), key players in cell-cell communication, have been widely explored for tissue engineering and regenerative medicine applications. Thus, the aim of this study is to assess the role of EVs derived from platelets in stem cell tenogenic commitment using a bioengineered tendon in vitro model for potential use as tendon therapeutic agents.

METHODS: Biomimetic platelet-derived EVs were produced by freeze-thaw cycles of platelets and isolation at different centrifugation speed (2). To recreate the architecture of tendons, a 3D system consisting of electrospun anisotropic nanofiber scaffolds coated with collagen encapsulating human adipose stem cells (hASCs) and different types of platelet-derived EVs, were produced. Then, the influence of the tendon-mimetic constructs and the distinct EVs populations in the hASCs tenogenic differentiation were assessed over culture time.

RESULTS: We observed that the hASCs on the nanofibrous tendon scaffolds, show high cytoskeleton anisotropic organization that is characteristic of tenocytes. Moreover, acting as biological cues, platelet-derived EVs boosted hASCs tenogenic commitment, supported by the increased gene expression of tendon-related markers (SCX and TNMD). Additionally, EVs enhanced the deposition of tendon-like extracellular matrix (ECM), as evidenced by the increased gene expression of ECM-related markers such as COL1, COL3, DCN, TNC, and MMP-3, which are fundamental for ECM synthesis and degradation balance. Moreover, EVs induced lower collagen matrix contraction on hASCs, which has been related with lower myofibroblast differentiation.

DISCUSSION & CONCLUSIONS: Overall, the results revealed that EVs are capable of modulating stem cells' behavior boosting their tenogenic commitment, through the increased expression of healthy tendon cell markers, potentiating ECM deposition and decreasing cell contractility. Therefore, platelet EVs are a promising biochemical tool, worthy to be further explored, as paracrine signaling that might potentiate tendon repair and regeneration.

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Keywords: Extracellular vesicles, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-250

Antibiotic and ion delivery from silica microspheres-based scaffolds for bone regeneration

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INTRODUCTION: Biomaterials are widely used to fabricate bone scaffolds with good bioactivity and mechanical properties. These properties can be conferred using organic/inorganic composites¹. Bioactivity can be introduced using bioceramics, which are mainly fabricated at high temperatures and are brittle. In this sense, silica microspheres (MSs)² are synthesized at low temperatures, are not brittle, and allow the incorporation of bioactive ions, such as Sr^{2+} . On the other hand, the use of gelatin as a support of the MSs will bring mechanical stability and serve as depot for the controlled release of other bioactive signals.

METHODS: Silica microspheres were synthesized by water in oil emulsion. They were added to gelatin, poured into molds and post treated by crosslinking and freeze-drying. The scaffolds were characterized by scanning electron microscopy (SEM) (morphology), weight loss, water uptake and compression tests. Sr^{2+} and rifampin (loaded in the gelatin matrix) release was studied by spectrophotometry. Cell adhesion, proliferation and differentiation (n=3) was analyzed with and without factors, using confocal microscopy, Pico green assay and Alkaline Phosphatase (ALP) activity tests.

RESULTS: SEM revealed the successful incorporation of silica MSs in the scaffold and an adequate porosity. Microspheres reduced the degradation of the scaffold and increased 4 times the mechanical properties. After the incorporation of Sr^{2+} and rifampin, we observed an initial release of the antibiotic and a delayed release of the ion. Cell studies showed that there was a better cell attachment, proliferation and differentiation after the incorporation of silica spheres. The incorporation of Sr^{2+} and rifampin accelerated the differentiation process.

DISCUSSION & CONCLUSIONS: The incorporation of MSs maintained porosity to allow cell migration. It also permitted the reduction of the degradation rate maintaining it constant along time. Mechanical properties were increased to better mimic bone matrix. The initial release of the antibiotic and the delayed release of the ion could avoid the infection and ensure the later regeneration. The presence of MSs increased cell attachment, proliferation and differentiation, which was enhanced by Sr^{2+} and rifampin.

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Keywords: Composite materials, Drug delivery

OP-251

Composite graded melt electrowritten scaffolds for regeneration of the periodontal ligament-to-bone interface

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INTRODUCTION: The interface between bones and connective tissues, i.e., ligament and tendon, is characterized by changes in cell phenotype and matrix composition and organization. These changes result in graded mechanical properties that allow the transmission of load without creating stress concentrations. Thus, regenerating such functionally complex interfaces is challenging and presents a serious clinical problem. One of the interfaces that is particularly challenging to regenerate is the bone-to-periodontal ligament (bone-PDL) interface. Here, collagen fibers form a firm connection between the tooth cementum and alveolar bone. Unfortunately, in the event of periodontitis, a common inflammatory disease, the bone-PDL interface gets damaged, which can ultimately result in the loss of the teeth. In this study, we hypothesize that a PDL microfibrillar scaffold that captures the graded composition and organization of native PDL tissue can induce stable, long-term, PDL regeneration.

METHODS: A custom-built meltelectrowriting (MEW) device was used to fabricate multi-layered constructs with different Magnesium Phosphate (MgP) – polycaprolactone (PCL) compositions and architectures. The effect of MgP (10, 20, 40wt%) on PCL MEW processing was systematically investigated in order to obtain reproducible fibre scaffolds with micrometer fiber sizes and high ceramic content. Bone forming potential of MgP-PCL fibre scaffolds was then evaluated in vitro over 28 days. After in vitro characterization, fabrication of graded constructs with different MgP compositions and architectures, cross-hatch and randomly oriented was investigated. Mechanical properties of printed scaffolds were characterized. Finally, most promising constructs were tested for their potential to promote stable bone-PDL interface regeneration in a periodontal rat model for 6 weeks.

RESULTS: We were able to reproducibly fabricate fiber scaffolds with micrometer fiber diameters ranging from 15µm - 40µm and 20wt% MgP content. Notably, MgP-PCL fiber scaffolds were able to promote in vitro bone formation in medium without osteo-inducing components. A three region scaffold with different highly porous boxed-shaped architectures and material compositions was successfully fabricated in one single print. A strong bond between PDL and bone region was observed. The micro-CT and histology analysis demonstrated that the MgP based composition and fiber scaffold organization were able to improve osseous repair and periodontal ligament regeneration in alveolar bone defects compared with SHAM and control groups.

DISCUSSION & CONCLUSIONS: In summary, we described the development of multi-region implants that could capture the graded composition and organization of native PDL tissue and in this way better promote PDL regeneration.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Ceramics

OP-252

Qiai essential oils-loaded graphene oxide-gelatin methacryloyl membrane for diabetic wound healing

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INTRODUCTION: The diabetic wound shows delayed and incomplete healing processes, which in turn causes infection, nerve damage, and even amputation. Biomaterial-based treatment with controlled release of signaling molecules is a promising method for diabetic wound healing. Gelatin methacryloyl (GelMA) has been widely used to develop biomaterial due to its good biocompatibility, water-absorbing capacity, and biodegradability. However, the persistent inflammation, oxidative stress, and bacterial infection in diabetic wounds restrict the further application of GelMA hydrogel. Traditional Chinese medicine has been increasingly recognized worldwide and extensively applied in the treatment of diabetes mellitus. Qiai essential oils (QEO), oily liquids extracted from Chinese medicine qiai by steam distillation, have been found to show favorable antioxidant, antimicrobial activity, and anti-inflammatory. Graphene oxide (GO) has been widely applied due to its unique physicochemical, antibacterial, and controlled release of bioactive agents. Therefore, we infer that it is possible to develop an excellent biomaterial-based hydrogel for diabetic wound treatment by constructing QEO-loaded GO-GelMA hydrogel.

METHODS: Physicochemical characteristics of QEO-GO-GelMA hydrogels were evaluated, including SEM, DLS, FTIR, Raman spectroscopy, swelling test, and rheology analysis. The antibacterial effects of QEO-GO-GelMA were investigated against two pathogenic strains of *E. coli* and *S. aureus*. What's more, the anti-oxidative stress, promoted proliferation, and accelerated migration effects on fibroblast were performed. Finally, the diabetic wound model was constructed, and the healing effects of QEO-GO-GelMA hydrogel were evaluated.

RESULTS: QEO-GO-GelMA hybrid hydrogel showed porous and interconnected structures. Moreover, the fabricated hydrogel exhibited favorable mechanical properties, water absorption, and swelling properties. Under near-infrared (NIR) light irradiation, the QEO-GO-GelMA hydrogel showed excellent antibacterial effects on *E. coli* and *S. aureus*. Furthermore, the fabricated hydrogel exhibited good biocompatibility, promoted cell proliferation, and accelerated cell migration. Finally, the hydrogel showed accelerated diabetic wound healing ability via the synergistic effects of QEO and GO.

DISCUSSION & CONCLUSIONS: The sustain-released QEO provided persistent anti-inflammation, anti-oxidative stress, and anti-bacterial infection in diabetic wounds. The incorporated GO provided a favorable drug-loaded platform and photothermal platform, which ensured the release of QEO and NIR-response anti-bacterial activity. This study provides new insights for developing biomaterial-based treatments for diabetic wound healing.

Keywords: Biomaterials, Wound healing

OP-253

Patient specific reconstruction of cleft palate deformities in dog patients with innovative 3D-printed organo-mineral cements

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INTRODUCTION: Developments in the field of additive manufacturing have allowed significant improvements in the design and production of scaffolds with biologically relevant features to treat bone defects. Unfortunately, the clinical and manufacturing workflow to generate personalized scaffolds is source of inaccuracies which lead to a poor fit between the implant and patients' bone defects. This may result in drastic consequences on the regenerative outcomes. Furthermore, most scaffolds display a non-adapted mechanical behavior, an inappropriate biodegradability rate and a mediocre potential to promote the formation of new vascularized bone tissues. Tackling these issues, organo-mineral scaffolds with evolutive mechanical properties were 3D-printed (3DP) to treat dog patients with spontaneous cleft lip/palate deformities.

METHODS: Patent about our discoveries is being filled; details about formulation and specific characteristics of 3DP scaffolds will be provided once intellectual property is validated.

Treatment of dog patients was approved by the French national and local ethic and welfare committee (CERVO-2022-14-V). To date 8 puppies with cleft palate/lip spontaneous deformities have been recruited and treated. Two weeks prior surgery, a live CT-scan of patient's skull was performed. Image segmentation allowed for the design of virtual 3D models of the defect and associated scaffold. For each patient, these virtual models were printed in polymer (PLA and TPU95 for the skull and scaffold, respectively) for surgical planning and validation. Organo-mineral pastes were formulated for further 3D printing with 25G cones. 3D-printing of scaffolds was performed with a BioX printer (CellInk); Pluronic F127 was used as support material. Surgical intervention implied the reconstruction of soft tissues, and the placement of the scaffold soaked with autologous bone marrow. Bone formation was followed 3 and 6 months after reconstruction, and a biopsy at 6 months was performed for more detailed analyses (μ CT, histology, SEM).

RESULTS: Scaffolds displayed great handling properties and were inserted within the bone defect without significant issue with a relevant bone edges/scaffold contact. Osteointegration of the scaffolds was observed after 3 months, and bone formation within the scaffold macroporous network seemed to occur.

DISCUSSION & CONCLUSIONS: Preliminary results have demonstrated an interesting potential of the set-up strategy to treat cleft lip/palate deformities in real spontaneous clinical setting. It also avoided the use of any irrelevant animal model; patients are going back to their owners after reconstruction.

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Keywords: Personalised medicine, Bone and bone disorders (osteoporosis etc)

OP-254

Heparin-guided binding of VEGF to supramolecular biomaterial surfaces to create dual-functionalized hemocompatible devices

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INTRODUCTION: A major challenge for cardiovascular biomaterials is creating an endothelial lining of the blood-material interface to prevent thrombus formation. A translational attractive approach to achieve this is in situ endothelialization via recruitment of endogenous cells. In this study, we aimed to create a hemocompatible and regenerative biomaterial by dual functionalization with anti-thrombogenic heparin and pro-angiogenic VEGF using supramolecular chemistry. To achieve this, we combined a heparin-binding-peptide (HBP) coupled to Ureidopyrimidinone (UPy), which is supramolecularly coupled to a UPy-modified PCL (PCLdiUPy) base polymer. Materials were functionalized with (1) a pre-complex strategy, in which heparin and VEGF165 were complexed before functionalization and (2) a two-step strategy, in which materials were first functionalized with heparin and next with VEGF165. By varying the functionalization strategy and the molecular ratio of heparin:VEGF165, a detailed understanding of the molecular adsorption on the surface and their influence on cell behavior was obtained.

METHODS: The materials were characterized by atomic force microscopy (AFM), water contact angle (WCA) measurements, and photoelectron spectroscopy (XPS). The adsorption behavior of both heparin and VEGF was assessed with quartz crystal microbalance with dissipation energy (QCM-D). The biological activity of the surface-bound VEGF was assessed based on the materials' capacity to support endothelial monolayer formation using human umbilical vein endothelial cells (HUVECs).

RESULTS: PCLdiUPy surfaces showed a fibrous morphology with phase separated domains observed upon addition of UPy-HBP, with a decrease in WCA. XPS showed that UPy-HBP addition increased surface nitrogen content, further demonstrating incorporation of UPy-HBP into PCLdiUPy. QCM-D showed a clear frequency shift upon heparin addition on UPy-HBP surfaces, depending on mol% of UPy-HBP, for both the two-step and the pre-complex functionalization approach. For the pre-complex approach, non-specific adsorption of the VEGF165 to the pristine PCLdiUPy surfaces was observed, while no significant shift was seen upon the flow of heparin. On UPy-HBP-presenting surfaces, HUVECs displayed higher cell coverage compared to heparin-only functionalized surfaces with a more favorable behavior on surfaces functionalized with the two-step approach. Also, on the UPy-HBP surfaces, the HUVECs displayed an enhanced surface coverage in response to heparin-VEGF functionalization when compared to the materials without the peptide, both for the one- and the two-step approach.

DISCUSSION & CONCLUSIONS: These findings confirm the efficient dual-functionalization of our material with a heparin-VEGF complex via a supramolecular HBP, and the bioactivity of the VEGF using HUVECs. This study forms the basis for a supramolecular biomaterial platform to enable modular functionalization with growth factor cocktails.

Keywords: Biologics and growth factors, Biomaterials

OP-255

Structurally patterned materials offer multiple options for guided cell behavior

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INTRODUCTION: Patterned materials allow to integrate multiple characteristics in a single material which better mimic the anisotropy found in tissues. We hypothesize that the matrix spatial patterning of biophysical cues can modulate cell behavior in terms of stem cell differentiation and collective cell and tissue organization. Therefore, this study aims to evaluate cell response in 3D alginate hydrogels with spatial patterns in biophysical and biochemical properties.

METHODS: The patterns on the material were formed using photopatterning and the combination of 2 crosslinking METHODS: MMP-sensitive peptide (Deg) and norbornene-tetrazine (nonDeg). The materials were mechanically characterized based on the elastic modulus using surface microindentation. Human mesenchymal stem cells (hMSCs) were encapsulated in 3D in patterned materials in hydrogels disks and cultivated under 3 different medias: growth, adipogenic and osteogenic. To evaluate cell behavior, cell viability (live/dead staining), cell morphology (DAPI/phalloidin), proliferative state (Ki67), mechanosensation (YAP) and differentiation were evaluated at days 1 and 14. Comparisons between hydrogels or time points were performed using Wilcoxon Signed Rank test ($p < 0.05$) using Origin Software.

RESULTS: The mechanical characterization showed comparable an elastic modulus in both phases (1-4kPa) The 3D encapsulation of human mesenchymal stem cells (hMSCs) in patterned materials showed high viability (>85%) over the 14 days in both phases and a significant increase in cell number in the degradable region (UV). Cell morphology showed a significant increase in cell area and decrease in circularity, indicating cell spreading in degradable areas. The effect of patterns showed differences proliferation marker Ki67, which has higher intensity in degradable zones. The expression of YAP was higher in the differentiation medias (osteogenic and adipogenic) compared to growth media. In growth media, the osteogenic differentiation was more pronounced in UV areas compared to nonUV. This effect of differentiation was more pronounced in osteogenic media, as mineralization starts in degradable areas whereas an inhibited differentiation is shown in non-degradable zones.

DISCUSSION & CONCLUSIONS: Patterned hydrogels were fabricated with spatially tunable mechanical and degradation properties determined by the crosslinking type. Differences in degradation properties influenced 3D hMSCs proliferation, morphology and differentiation. Ongoing work is investigating the correlation between YAP/TAZ localization and its role in cell response.

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Keywords: Biomaterials, Differentiation

OP-256

Chemotherapeutic Screening on a Scaffold based Dynamic *In Vitro* Multicellular Models Pancreatic Cancer

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INTRODUCTION: Pancreatic Ductal Adenocarcinoma (PDAC) is reported as the 7th leading cause of cancer related deaths worldwide. This is partly attributed to the tumour microenvironment and its resistance to treatment. Advanced study of the disease and its treatment resistance mechanisms require the development of biomimetic *in vitro* tumour models. We have previously developed poly-urethane (PU) based pancreatic cancer model using (i) pancreatic cancer cells (monocellular model) and (ii) pancreatic cancer cells, stellate cells and endothelial cells (multicellular model). We have shown long term physiological maintenance, feasibility of mimicking extracellular matrix (ECM), mimicry of PDAC fibrosis and have mapped effect of hypoxia (1-4). Although interstitial fluid flow is an important parameter in the development of cancer niche models and therapeutic assessment, studies involving the role of this critical parameter for PDAC is lacking.

In the current work we introduce dynamic flow (interstitial fluid flow mimicry) in our multicellular models and perform for the first time a comparative chemotherapeutic assessment in static and dynamic conditions.

METHODS: PU scaffolds were prepared and coated for ECM mimicry as previously described (1-4). A zonal structure with (i) endothelial and stellate cells on the outer scaffold coated with collagen I and (ii) pancreatic cancer cells in the inner scaffold coated with fibronectin was designed, along with a single scaffold based multicellular model ('cell cocktail' approach) (4). Dynamic flow was introduced with a perfusion bioreactor. 50µM Gemcitabine (GEM) was applied to all models. Imaging of cellular proliferation/spatial organisation and ECM secretion was carried out along with q-PCR assessment of various biomarkers.

RESULTS: We observed that the zonal multicellular model of PDAC showed a higher resistance to GEM in comparison to the single scaffold assisted multicellular model. Furthermore, dynamic flow affected the cell spatial organisation, metabolic production, biomarker expression and chemotherapy response.

DISCUSSION & CONCLUSIONS: The results demonstrate the importance of spatial architecture mimicry and its role is chemotherapeutic resistance for pancreatic cancer *in vitro* tissue model. In addition we have also demonstrated that dynamic fluid flow is key player such models. Our developed model is a novel, complex biomimetic low-cost high throughput tool that can be used for personalised treatment screening of pancreatic cancer

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Keywords: Bioreactors, Disease models

OP-257

A 3D Bioprinted Biphasic Model of the Intervertebral Disc with Regional Cell Morphology and Collagen Alignment

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INTRODUCTION: The intervertebral disc (IVD) aids the flexible movement of the spinal column¹. It is comprised of two distinct tissue regions. The gelatinous, polysaccharide-rich nucleus pulposus (NP) is populated by spherical cells. Conversely the stiffer, outer annulus fibrosus (AF) is comprised of elongated cells in an aligned, fibrous collagen-rich matrix². Disc degeneration is common with aging. Understanding the disease processes is often impaired by a lack of translatable in vitro models. This study aims to utilise suspended layer additive manufacturing (SLAM)³ to fabricate IVD analogues with accurate AF architecture and an NP-AF interface for disc disease modelling.

METHODS: A biphasic hydrogel construct was fabricated via SLAM. Constructs were generated containing regions of gellan gum (gellan) and type I collagen to recapitulate the NP and AF regions respectively. Human NP and AF cell lines were incorporated into the relevant regions of the construct and cultured for 28 days. The printed constructs were analysed with atomic force microscopy (AFM) to evaluate gel microstructure and biomechanics. Phalloidin staining was used to evaluate cell morphology at d7-28. Additionally, cell tracking dyes were employed to evaluate cell migration within the construct and immunofluorescence was utilised to screen for extracellular matrix depositions over 28 days of culture

RESULTS: Constructs were successfully fabricated containing two distinct gel phases to represent the NP and AF. Analysis of the microstructure with AFM revealed that the bioprinting of the tissue analogue facilitated the generation of aligned collagen fibres in the AF and an ordered polysaccharide network in the NP. Live cell tracking demonstrated the successful incorporation of NP and AF cells into the two tissue regions. The embedded cells also exhibited distinctly different cell morphologies with spherical NP cells, elongated AF cells and an observable NP-AF cell interface. Actin staining of the embedded cells revealed the presence of aligned AF cell populations that oriented in the same direction as the observed aligned collagen fibres. Fluorescence staining also highlighted the deposition of hyaluronic acid (HA) by NP cells into the construct over 28 days.

DISCUSSION & CONCLUSIONS: These data highlight the potential to utilise SLAM for the generation of constructs for IVD disease modelling applications. This model will now be utilised to study IVD-like tissue formation mechanisms and the influence of microenvironmental factors such as hypoxia, mechanical load and ECM composition on IVD degeneration.

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Keywords: 3D printing and bioprinting, Intervertebral disc / spine and their disorders

OP-258

Bio-fabricated Heart-in-a-dish models for transplantation and drug development

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INTRODUCTION: Chronic heart failure accounts for 64.3 million patients worldwide, 1 million of which account for UK citizens. Heart attack results in irreversible loss of cardiomyocytes leading to heart failure with a 50% 5-year mortality. Chronic heart failure currently does not have a cure or effective treatment and is managed with drugs to slow the process. Cell-free cardiac patches approved for clinical use in the USA are unable to effectively prevent fibrotic scar tissue formation which occurs after heart failure and cannot replace lost cardiomyocytes which are unable to replicate. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), offer a reproducible and sustainable resource of cells. However, a significant challenge lies around identifying physiologically relevant models for cardiovascular disease (CVD) modelling and cellular therapeutic applications. Here we explore different models for understanding the complexities of the heart.

METHODS: The chemo-topography model (polymer coated micropatterned surfaces), identified from screening the TopoChip¹ (~2000 micro-topographies) and polymer² (~300 polymer chemistries) micro-arrays have been fabricated using hot-embossing techniques to produce polystyrene inserts or PRIMO (alveole) maskless photopatterning onto well-plates for drug development. Mini-hearts created using biofabrication printing techniques combine stromal supportive cells of the heart with hiPSC-CMs to recapitulate tissue architecture. Heart-in-a-dish platforms have been used to investigate differences between healthy and diseased hiPSC-CMs assessed by their contraction using CelloptiqTM and structural interactions quantified using artificial intelligence guided image analysis and custom sarcomere banding analytical tools.

RESULTS: Micro-topographies improved structural integrity of hiPSC-CMs by promoting cell elongation (aspect ratio 6-7) and alignment (sarcomere organisation) whilst the discovered proprietary polymer coating improved hiPSC-CM function by increasing contraction amplitude and beat rate (~60-70 beats per minute). The combinatorial technologies can improve multiple maturation factors for better hiPSC-CM modelling. Small-scale drug screening assays indicate improved toxicity assessments for drug development. Phenotypic differences between healthy and diseased were identified in stromal cells which in 3D recapitulated fibrotic processes following a heart attack when combined with hiPSC-CMs.

DISCUSSION & CONCLUSIONS: The fully-synthetic "chemo-topography" platform completely eliminates the need for common animal-derived coating currently used to support hiPSC-CM attachment, reducing cost by ~300 fold. We expect this to benefit both supply as well as application

of hiPSC-CMs which include drug cardiotoxicity screening. Scale-up of tissue-like mini-heart structures are more physiologically relevant for CVD modelling, understanding chronic heart failure and creating prototypes for implantable heart patches.

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Patel AK Biomaterials. et al 61:257-65, 201

Keywords: Biomaterials, Cardiovascular

OP-259

Developing 3D Peripheral Nerve Injury models for Long term analysis of Materials

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INTRODUCTION: Traumatic peripheral nerve injuries (PNI) are common, with 9000 cases in the UK and 200,000 cases in the USA annually. Functional recovery is often poor and even with current surgical interventions more than half of the patients still develop persistent pain¹. Nerve guidance conduits (NGCs) are used to expedite and improve the healing process. However clinical outcomes with NGCs are often poor as “inert” materials lack the biological, physical and chemical signals required for nerve regeneration. Testing new materials/cellular therapies for nerve repair often requires 2D in vitro testing on flat surfaces, in static culture, that does not represent the in vivo environment, prior to in vivo work using small animal models.

METHODS: Polycaprolactone (PCL) fibres, manufactured via electrospinning, were threaded into 3D printed PCL NGCs. Embryonic Chick Dorsal Root Ganglion (DRG) bodies were extracted from spinal columns, and placed onto fibres in a 3D ex vivo testing model². DRGs were exposed to static conditions as well as dynamic conditions (50 μ L/min), using the Kirkstall QuasiVivo 900 (QV900) multi-chamber perfusion system, for 7 – 21 days to assess axon outgrowth length, Schwann cell migration length and Schwann cell myelination by immunolabelling for β III tubulin, S100 β , myelin basic protein and myelin protein zero respectively.

RESULTS: Aligned fibres of PCL were successfully manufactured using electrospinning and threaded into PCL 3D printed NGCs. Fibre alignment through conduits was confirmed via microCT. Dynamic conditions significantly supported axon outgrowth and Schwann cell migration distances from chick DRG explants after 7 days in culture, compared to static conditions alone.

DISCUSSION & CONCLUSIONS: The addition of flow significantly supports neuronal and Schwann cell viability, differentiation and myelination compared to static culture. The addition of flow has been shown to more closely mimic the in vivo environment, allowing the testing of a multitude of materials and decrease the number of animals required for in vivo testing.

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Keywords: Nervous system (brain-central-peripheral / disorders), Bioreactors

OP-260

Directed Differentiation of Human iPSCs into Mesenchymal Lineages by Optogenetic Control of TGF- β Signaling

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INTRODUCTION: TGF- β signaling modulates key mesenchymal stem/stromal cell (MSC) functions. Despite numerous studies on TGF- β signaling in MSCs, the results are inconclusive, in part due to inherent batch variability, inconsistent culture stability, and lack of spatiotemporal control over soluble factors. Optogenetics can offer unprecedented precision and dynamic control of cell signaling using light.

METHODS: We report the development of an optogenetic human induced pluripotent stem cell (hiPSC) based system for TGF- β signaling and demonstrate its utility in driving TGF- β mediated mesenchymal differentiations. We generated stable hiPSC lines expressing optogenetic TGF- β receptors which maintained normal karyotype and pluripotency. Following differentiation into MSCs (optohiMSCs), cells continued to express the optogenetic receptors and met all standard criteria for MSC characterization.

RESULTS: In 2-week smooth muscle and tenogenic differentiations from optohiMSC monolayers, gene and protein level expression of the respective markers (alpha smooth muscle actin, calponin-1; collagen I, scleraxis) were significantly increased in groups which had TGF- β signaling activated by either soluble factor or optogenetic stimulation, compared to groups which received neither. Optogenetic stimulation proved effective in 3D as well, as evidenced by global quantitative proteome analysis after 3-week chondrogenic differentiations, with samples from 2 independent experiments. Hierarchical clustering on a heat map of relative protein expression showed that in each experiment, groups which received optogenetic stimulation had protein expression profiles more similar to the soluble TGF- β treated positive control group than the untreated negative control group which received neither soluble factor nor optogenetic TGF- β stimulation. To demonstrate an advantage of using the optogenetic system rather than soluble factors to manipulate signaling, non-optogenetic and optogenetic hiMSCs were mixed in co-culture using shared culture medium. By selectively activating TGF- β signaling with light, we maintained both a stromal/stem-like population as well as a differentiated smooth muscle-like population in a single culture.

DISCUSSION & CONCLUSIONS: The simultaneous maintenance of stromal support cells and their differentiated progeny could be used to study biological scenarios such as the plasticity of MSC-like pericyte vascular support cells to differentiate into vascular smooth muscle cells in arteriogenesis, or MSC recruitment to sites of bone injury to replace damaged osteoblasts. The collected data suggest that optogenetic regulation of TGF- β signaling can enable spatiotemporally precise control of hiPSC differentiation into mesenchymal lineages, towards more comprehensive understanding of the role of TGF- β in regulating MSC function.

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Keywords: Biologics and growth factors, Differentiation

OP-261

Melt electrowriting of magneto-active fiber scaffolds for skeletal muscle cell stimulation *in vitro*

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INTRODUCTION: Current therapies against muscular dystrophies do not generally address losses in myotube alignment that support muscle contraction. Moreover, mechanical stimulation of injured skeletal muscle alone has shown to facilitate functional regeneration *in vivo*. Here, we hypothesized that magneto-active scaffolds with well-organized fiber architectures guide myotube growth and remotely stimulate muscle contraction. To achieve this, melt electrowriting (MEW) —a unique technique for fabricating highly ordered microfiber scaffolds [1]— was performed from polycaprolactone (PCL) blends with iron oxide nanoparticle (ION)-modified partially reduced graphene oxide nanoplatelets (rGNP), generating magneto-active scaffolds that support muscle cell growth.

METHODS: Medical-grade PCL was melt-blended with ION-decorated rGNP (rGNP@), yielding magneto-active composites (rGNP@ content of 2–20wt.%). Their processing was systematically assessed based on key MEW parameters in a custom device [1]. Well-organized fiber scaffolds with hexagonal micro-structures were fabricated, and their assembly into cartesian or polar grid macrostructures was explored. The effect of rGNP@ content and MEW parameters on scaffold morphology, magnetic properties, and mechanical performance was studied.

MEW/Matrigel/collagen scaffolds were seeded with C2C12 immortalized mouse myoblasts. Cell viability, differentiation efficacy, and fusion were studied after 1 and 8 days in culture.

RESULTS: ION deposition on rGNP yielded well-dispersed rGNP@ particles with sizes above 0.5µm and low aspect ratio. Homogeneous PCL/rGNP@ composites with high magnetic filler content (up to 20wt.%) were obtained by solvent-free melt-blending. MEW enabled the design of magnetic hexagonal scaffolds with tunable fiber diameter, modularity, and zonal distribution of magneto-active and nonactive material. Hexagonal scaffolds (400µm thickness, 600µm pore size, fiber diameter: 18–22 µm) displayed elastic deformability under tension, mitigating limitations due to high filler content. External magnetic fields below 300mT triggered out-of-plane reversible deformation in scaffolds with filler content above 10wt.%. Moreover, myoblast culture on Matrigel/collagen/MEW scaffolds showed that magnetic particles did not significantly affect viability after 8 days. Under static conditions, myoblasts underwent similar differentiation rates regardless of the presence of magnetic particles.

DISCUSSION & CONCLUSIONS: Highly ordered microfiber scaffolds based on PCL/rGNP@ blends were reproducibly fabricated by MEW, enabling controlled deposition of zonally distributed magneto-active materials with custom geometries. This allowed the design of actuating scaffolds that support skeletal cell growth and differentiation *in vitro* and magnetically triggered mechanical stimulation, providing new perspectives towards consistent soft-robotic skeletal muscle microtissues *in vitro*.

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Keywords: 3D printing and bioprinting, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-262

A novel platform for 3D electrical stimulation of GBM-based scaffolds for neural tissue engineering

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INTRODUCTION: Given the relevance of neural tissue pathologies and their severe consequences, different approaches have shown potential for a future solution. This work focuses on three of those approaches that together can create an integrative solution: a) adipose-derived extracellular matrix (adECM) scaffolds, b) graphene-based materials (GBM) and c) electrical stimulation (ES). adECM is here exploited as an abundant and easily available source of proteins long-known as suitable platforms for cell growth. GBM have shown both in vitro and in vivo to promote axonal outgrowth, while exogenous electrical fields have shown to maintain neural activation promoting pathways and pull and bypass axons through damaged areas.

METHODS: In this work, graphene multifunctionality is exploited to fabricate a novel 3D ES device that allows current flow across scaffolds during culture. Graphene electrodes were screen-printed and integrated into culture wells. A thorough analysis of the electrode's impedance with time and frequency was performed to optimize the 3D device working conditions. Additionally, an interface for in vitro ES was created with versatile hardware and software that allows a wide range of stimulation parameters. The graphene-based 3D ES platform was tested with neural stem cells seeded on scaffolds based on GBM and adECM with the following freeze-dried compositions: adECM and adECM/GBM (50/50 w/w). The scaffolds were electrically stimulated with a 10 Hz bidirectional current signal of 200 μ A for 1 hour every day during 14 days.

RESULTS: All the 3D ES platform parts are suitable for culture and the exposure of RAW-264.7 macrophages to electrodes' GBM ink did not induce oxidative stress nor polarization toward the

proinflammatory M1 phenotype. Even though GBM-containing scaffolds showed less cell adhesion, more DNA content was measured at the last culture day. More DNA was also found in electrically stimulated scaffolds, especially significant in GBM-containing scaffolds where ES induced 1.65-fold increase ($p < 0.05$) in proliferation. Non-stimulated and stimulated scaffolds exhibit Tuj-positive cells, specific to neuronal differentiation. While the non-stimulated scaffolds exhibit clusters of cells with no significant neurite's formation, these 3D stimulated scaffolds revealed specifically thick and long ones between neighbouring neurospheres. Also, synaptophysin was highly expressed in the stimulated scaffolds.

DISCUSSION & CONCLUSIONS: This work highlights the potential of graphene multifunctionality with a novel biocompatible graphene-based 3D electrical stimulation device. A proof of concept is provided with the enhanced proliferation and differentiation of neural stem cells in graphene-based scaffolds.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Enabling technologies

OP-263

Photo-switchable bio-interfaces for dynamic cell cultures

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INTRODUCTION: Cellular microenvironment carries out a crucial role in affecting and dictating cellular functions thus influencing several biological processes [1]. The bidirectional communication between cells and the extracellular matrix (ECM) occurs through the transmission of biochemical/biophysical signals at sub-micrometric level involving different sub-cellular component such as focal adhesions, cytoskeleton structures and the nucleus. [2]. The aim of this work is to realize a dynamic cell culturing platform, by exploiting the photo-switchable properties of azobenzene molecules, which allow to precisely modulate surface topographic features both in space and time.

METHODS: Poly(disperse red 1 methacrylate) (pDR1m) was spin coated to realize a thin film on glass bottom petri dishes and a sub-micrometer grating was inscribed by using a scanning confocal microscope with 514 nm wavelength. The removal of the impressed pattern was performed by using a mercury lamp. A JPK NanoWizard II was used to measure mechanical properties of living cells. MCF10A cells were grown in DMEM/F12 with 5% horse serum. Cells were seeded on pDR1m substrates at 10^3 cells/cm² 24h before pattern inscription. Then, cells were fixed in paraformaldehyde 4% and immuno-stained for paxillin, nuclei and actin. Focal adhesions, cells and nuclei area, morphological parameters were measured by Fiji. Nuclear volume was measured by Imaris.

RESULTS: The dynamic azopolymer platforms were realized to provide cyclic topographical signals to cells. Results showed the efficacy of the dynamic system to modulate cell and focal adhesions morphology such as area, elongation and orientation. Moreover, MCF10A cell nuclei were also affected, with nuclear area, elongation and volume increasing from flat-to-pattern and decreasing after pattern-to-flat variations, resembling the flat condition. Finally, cell mechanical properties showed an increase of the cell Young's modulus on pattern and a decrease on pattern-to-flat surfaces.

DISCUSSION & CONCLUSIONS: Topographical signals are known to affect cell behavior at different levels, from adhesion to differentiation. Here, a dynamic photo-switchable platform was realized to transmit cyclic stimuli and to give precise instructions to cells thus controlling their functions. Indeed, the ability of azobenzene molecules to induce a reversible surface mass transport has led to the realization of a cell culturing platform for the investigation of cell behavior in a dynamic context. The transmission of specific instructions paves the way to the possibility to control and revert cell functions both in space and time.

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Keywords: Biomaterials, Interfaces - engineered

OP-264

electrical stimulation positively interferes on induced pluripotent stem cell neural differentiation at different stages

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INTRODUCTION: Patient derived Induced pluripotent stem cells (iPSCs) are a promising source for reliable in vitro models for disease study and future transplants. This is critical for neurological diseases, as lack of effective therapies push researchers to develop reliable models. Electrical stimulation (ES), a powerful biophysical cue, can be applied to both improve cell differentiation and be used as future therapy. The goal of this work was to study the effect of ES on neural-differentiating iPSCs.

METHODS: Setups capable of ES were developed using electroconductive biomaterials, including 1) biodegradable and electroconductive poly(aniline):Camphorsulfonic-acid doped poly(caprolactone)/poly(glycerol sebacate) (PCL-PANI:CSA/PGS) coaxial electrospun fibers and 2) highly conductive acid-treated spin-coated poly(ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) films. We performed material characterization and evaluated in vitro performance. Both naïve iPSCs and induced neural progenitor cells (iNPCs) were seeded on the materials and promoted to differentiate into the neural lineage under ES.

RESULTS: PCL-PANI:CSA/PGS fibers were produced. Average diameter was 951 ± 465 nm and electroconductivity 0.063 ± 0.029 S cm⁻¹. The mechanical properties ($\epsilon = 1.3$ MPa) and hydrophilicity (38 °) favor neural cell culture. Electrical stimulation induced an up-regulation of numerous neuron markers, including glutamatergic (15-fold) and voltage-sensitive channels (12 for SCN1A, 32-fold CACNA1C) and down regulation of GABAergic marker (11 fold).

Spin-coated PEDOT:PSS films were treated with sulfuric acid to yield highly conductive substrates (≈ 4000 S cm⁻¹). Acid treatment improved film stability for 30 days in PBS, affected surface morphology but did not affect the adsorption of Poly(ornithine)/Laminin and Matrigel to its surface. Proliferating iPSCs maintained their pluripotency for 7 days of culture and were able to differentiate into iNPCs. Selective ES application improved neural differentiation, with long and parallel axons emerging at day 17 from neural rosettes and improved expression of FOXG1 and Tuj1 neural markers.

DISCUSSION & CONCLUSIONS: Our results demonstrate that ES positively influences neural-differentiating iPSCs and iNPCs alike. Moreover, selective application of ES at different stages of differentiation dramatically improved cell phenotype in different fashions. Evaluation of the full-length protocol using different electroconductive biomaterial approaches is now possible. These results pave our future work aiming at full-organ regeneration.

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Keywords: Nervous system (brain-central-peripheral / disorders), Electroactive materials

OP-265

The High C: Collagen Deposition Under Combined Vibrational And Tensile Load In Adipose-Derived Mesenchymal Stromal Cells For Vocal Fold Tissue Engineering

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INTRODUCTION: Among many areas in the human body that are exposed to mechanical stress, the vocal folds (VF) represent a particularly multi-stressed tissue undergoing tensile, shear, compressive, frictional as well as other fluid- and aero-dynamic forces. In VF scarring, the extra-cellular matrix (ECM) of the tissue is disorganized, resulting in disturbed vibration and a hoarse voice, which is sometimes even followed by life-threatening laryngeal obstruction. Treatment options for VF scarring are clinically challenging and limited, as up to date there is no efficient strategy to restore the complex VF microstructure and thereby the tissue's biomechanical properties. In most cases, the therapy focuses on the maintenance of essential respiratory functions. For this reason, cell therapies, injectable hydrogels as well as tissue engineering solutions have moved into the focus of research in order to address the high clinical need. In this study, we used a custom-made bioreactor system to investigate the impact of combined vibrational and tensile forces on tissue-engineered VF constructs *in vitro*. Moreover, we analyzed the influence of the stimulation on ECM production and orientation.

METHODS: Adipose-derived mesenchymal stromal cells (ASC) were seeded on collagen-elastin-based membranes and cultured statically or dynamically for up to 3 days. 20 % tensile strain were combined with 200 Hz vibration frequency in order to mimic regular human phonation. We used alternating stimulation patterns with resting periods. After cultivation, cell survival as well as deposition and orientation of ECM components (mainly collagens and elastin) were evaluated using live-dead-staining as well as immunohistochemical and histological means. Furthermore, we analyzed the Young's moduli of the tissue-engineered constructs after cultivation under static and dynamic conditions.

RESULTS: ASC survived dynamic cultivation under combined mechanical load over different periods of time. Additionally, we could show an increase in production of collagens and elastic fibers after dynamic culture in comparison to static samples. Mechanical testing revealed differences in Young's

moduli depending on cultivation method and time period.

DISCUSSION & CONCLUSIONS: Our results provide new insights for the *in vitro* regeneration of VF tissues using mechanobiological stimulation. In the future, our bioreactor system can contribute to the development of a VF replacement tissue and furthermore assist in understanding the remodeling processes of VF that undergo pathophysiological mechanical stresses.

Keywords: Bioreactors, Multipotent (mesenchymal) stem cells

OP-266

Investigation of periodontal ligament stem cells exposed to controlled mechanical and electrical stimulations in view of periodontal ligament tissue engineering

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INTRODUCTION: Periodontal ligament (PDL), connecting bone and tooth root, is crucial for mechanical stabilization and mastication-related forces absorption. These capacities deteriorate if PDL is affected by periodontitis, a widespread degenerative disease that can lead to tooth loss. Tissue engineering could represent a promising strategy for treating periodontitis; however, due to the PDL complexity, any regeneration strategy should entail studying specific signals that guide the formation and remodeling of the PDL¹. The aim of this study was to investigate the response of human PDL-derived stem cells (hPDLSCs) to controlled mechanical and electrical stimulations.

METHODS: hPDLSCs from healthy donors and adipose-derived stem cells (hASCs, ASC52-telo hTERT, ATCC), selected as comparative cells, were exposed *in vitro* to mechanical or electrical stimulations. For applying uniaxial stretch, a stretch bioreactor² combined with flexible substrates was adopted. For each cell type, the substrates were coated with collagen I (Sigma Aldrich), seeded with cells (2x10⁵ cells/substrate), statically cultured for 6 hours, and moved to the bioreactor for 3 days under uniaxial stretch (8% pre-strain + 7% cyclic strain for 90s, 1Hz, every 6h; n=3). Cell-seeded substrates were cultured statically as control (n=3). For delivering electrical stimulation, the electrical stimulator ELETTRA in combination with custom culture chambers³ was adopted. hPDLSCs (n=5) and hASCs (n=3) were separately seeded in culture chambers (1x10⁵ cells/well), statically cultured overnight, and then exposed to pulsed electric field (4V/cm, 1Hz) for 5 days. Non-stimulated cells were cultured as control (n=3). Osteogenic markers (ALP, OCN, RUNX2, COL-I) and P2X7R (for electrical stimulation) were analyzed by real-time PCR.

RESULTS: Real-time PCR analysis revealed that stretched hPDLSCs, in comparison to the control, showed an increasing trend of osteogenic marker expression, with a significant increase of OCN. Differently, stretched hASCs showed no significant changes. Concerning electrical stimulation, no significant differences were observed between stimulated and statically cultured hPDLSCs, while electrical stimulus induced an increasing trend in the expression of osteogenic markers and P2X7R in hASCs.

DISCUSSION & CONCLUSIONS: hPDLSCs and hASCs responded differently to applied physical stimulations. Uniaxial stretch promoted osteogenic response in hPDLSCs with no specific response in hASCs. While pulsed electric field increased the osteogenic expression of hASCs, without significant effects on hPDLSCs. Further biological tests imposing different stimulation protocols and using pathological hPDLSCs are ongoing, with the final aim to assess the potential of physical stimulations

for PDL tissue engineering approaches.

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Keywords: Stem cells - general, Biomechanics / biophysical stimuli and mechanotransduction

OP-267

High Cell Density Gel Co-Cultures and Cell-Fibre-Gel Composites via Reactive Jet Impingement Bioprinting

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INTRODUCTION: Reactive jet impingement (ReJI) is a bioprinting process which combines droplet streams of polymer and crosslinking solutions to create hydrogel droplet streams [1]. If cells are suspended in either or both of the starting solutions then cell-filled-gel droplets are created, with the capability to produce high cell density gels (up to 40 million cells/mL of gel). This paper illustrates how the process can print gel droplets onto rough and delicate substrates, such that cell-filled-gels can be deposited onto other gels, fibrous substrates [2], hard substrates [3], and tissues in order to create a wide variety of tissue cultures and models.

METHODS: ReJI bioprinting was performed with a three-component collagen-alginate-fibrin (CAF) gel which has been previously described [1-4]. Cell-filled-gels were deposited at a range of concentrations (from 4 to 40 million cells/mL of gel). Lamellar osteochondral co-cultures and cell-fibre-gel composites were produced and cell-filled-gels were directly printed onto 3D printed titanium alloy (Ti6Al4V), porous apatite-wollastonite (AW) surfaces and liver tissue (PCLS [5]).

RESULTS: The printed cell filled gels showed high cell viability on all substrates, and in all cases the high cell densities meant that cells would interact with the substrates that they had been deposited on. High cell densities produce significant extracellular matrix (ECM) as the cultures mature, producing biomimetic cell-cell and cell-ECM interactions. In fibroblast-gel-fibre composites higher cell densities promoted faster cellular organization. HCC cells printed onto PCLS within a co-culture are able to migrate from the gel into the liver tissue, offering potential as a model of cancer cell invasion.

DISCUSSION & CONCLUSIONS: The ReJI bioprinting system offers a versatile way to produce high cell density cultures, and to deposit these on a wide range of substrates. Cells are localised and produce high levels of ECM but can also effectively interact with complex substrates to produce microtissues for physiological models and regenerative medicine applications.

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Keywords: Biofabrication, Interfaces - engineered

OP-268

Acoustic patterning of microcapillary networks for 3D vascularized *in vitro* models

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INTRODUCTION: Cm-scale *in vitro* models require vascularization to improve physiological conditions. Fibrin has been largely used as hydrogel for conducting endothelial cells (EC) toward angiogenesis and vasculogenesis processes. Usually, EC are homogeneously dispersed within fibrin in small-scale microfluidics chips. Thus, they create random capillary network, lacking the anisotropic and hierarchical organization of the native vasculature. Among the biofabrication technologies, Sound Induced Morphogenesis (SIM)¹⁻² uses sound vibrations to generate hydrodynamic waves for contactless assembly cell suspension into spatially organized patterns. Here we show large scale patterning of microcapillary networks via SIM in a fluidic chamber for 3D vascularized *in vitro* models.

METHODS: In a custom-made platform, EC and stromal cells are assembled over 350 mm² surface area in concentric circles or honeycomb geometries in short time (<2 minutes) and characterized by fluorescence image analysis. Moreover, a perfusable channel of 900 µm in diameter is created in a fibrin layer superior to the microcapillary network, which is lined with EC. The stratified fibrin structure hosting capillaries and perfusable channel is closed with a glass coverslip, obtaining a large, perfusable, and vascularized construct. Osteoblast cells are introduced in the chamber as single cells or spheroids formulation.

RESULTS: After 7 days in culture, anastomosis occurs between the perfusable channel and the microcapillary network. The condensed areas of the pattern geometries measure 1023 ± 43 µm in thickness and are populated with capillaries which kept the patterned configuration over the days in culture. We proved that stromal cells support the maturation of vascular patterns which was quantified by immunofluorescence staining of vascular endothelial cadherin and lumen measures. In addition, the osteoblasts introduced into the chamber resembled vascularized bone tissue. Our results demonstrate the biofabrication of a geometrically versatile platform for large scale vascularized and perfusable *in vitro* models.

DISCUSSION & CONCLUSIONS: We anticipate that more relevant tissue models could be realized integrating hierarchically organized and reproducible vascular patterns with other tissue cells. The presence of perfusable channels allows nutrients exchange and interstitial flow. Altogether this approach could lead to generate morphologically relevant *in vitro* models to resemble closer the native tissues.

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Keywords: In vitro microenvironments, Vascular systems / vascularisation and heart

OP-269

Pancreatic ECM-based microencapsulation as an insulin delivery system for diabetes therapy

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INTRODUCTION: Pancreatic islets microencapsulation has been long studied as a diabetes therapy, thus isolating and supporting the encapsulated islets post-transplantation. The material used for microcapsules fabrication, however, has a crucial role in mimicking the natural tissue and granting the islets structural, biological, and mechanical support. Therefore, we developed a microencapsulation platform based on the biocompatible bioactive porcine pancreatic extracellular matrix (ppECM), hypothesizing that it will provide the islet with a natural pancreatic niche.

METHODS: Porcine pancreatic ECM was decellularized and enzymatically solubilized to produce a thermally induced ppECM hydrogel, which was further applied to microencapsulate rat islets using electrospray technology. ppECM encapsulated islets were characterized for their viability (FDA/PI), glucose-stimulated insulin secretion (2.8mM vs 16.7mM), and recovery after hypoxic conditions (2% oxygen). To evaluate the in-vivo safety of this platform, encapsulated islets were injected subcutaneously into C57BL mice and retrieved at 1 and 4 weeks post-encapsulation. Microcapsule integrity and fibrotic overgrowth on the microcapsule surface, as well as cell viability and morphology, were evaluated, and blood was tested for a complete blood count (CBC). The efficacy of our platform, in terms of blood glucose level correction, was addressed in immunocompetent diabetic mice.

RESULTS: ppECM microencapsulated rat islets remained viable for at least 14 days and demonstrated proper function, expressing insulin and glucagon. Moreover, their insulin secretion was shown to be glucose-responsive and on day 14 post-encapsulation, it was significantly higher than control-encapsulated islets. Furthermore, β -cells in the ppECM and the control-encapsulated islets expressed Pdx1, which is required for their maintenance and survival in the mature pancreas. The preservation of the islet structural integrity was demonstrated by the islet expression of Collagen IV. The protective effect of the ppECM under hypoxic conditions was shown through their complete recovery 1 week after hypoxia induction. The microcapsules' biocompatibility was demonstrated in-vivo, showing a similar profile of CBC to controls 1 and 4 weeks post-transplantation with no significant inflammatory reaction.

DISCUSSION & CONCLUSIONS: ppECM-based microencapsulation supplies the encapsulated rat islets with a natural bioactive niche that promotes their long-term survival and function as well as their recovery after stress conditions. Moreover, The isolated niche provides the islets with immunological protection when transplanted into immunocompetent mice. Altogether, these findings demonstrate the potential of ppECM-based microencapsulation as a prospective insulin delivery system for the treatment of diabetes.

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Keywords: Decellularised matrices, Drug delivery

OP-270

An engineered "in vitro" osteochondral tool to study osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a degenerative pathology that affects a large size of population and leads to significant impairment of the quality of life and disabilities. OA is characterised by the destruction of the articular cartilage (AC), inflammation of synovial membrane and remodelling of the subchondral bone [1;2]. The increase of catabolic protein production by several joint cells is followed by the action of stimuli such as cytokines or chemokine [3]. Several studies deepening the knowledge of the onset of OA and its treatment have been carried out, but there are still uncertainties about the molecular pathways involved, as well as the long-term treatment [4]. In this work, a biomimetic in vitro model of osteochondral human tissues was developed to recapitulate healthy and pathological features to offer a new tool to improve OA molecular investigations and pharmacology studies.

METHODS: For the subchondral bone, a polylactic acid (PLA) trabecular-like structure was 3D printed and functionalized with gelatin (Gel) and hydroxyapatite (nHA) through immobilisation via poly-dopamine (DP) coating. For the AC deep layer zone, a photocurable Gellan Gum (GG; 3%w/v) hydrogel structure with channels was obtained via soft-lithography and attached to the 3D PLA scaffold before the GG crosslinking. Immortalised mesenchymal stem cells (Y201s) were seeded on PLA scaffold, while chondrocytes differentiated from Y201s (Y201-Cs) [5], were encapsulated in each channel of GG layer within a chondroitin sulphate-based hydrogel. OA milieu was modelled by cytokines (IL-6, IL-1 β and TNF- α) addition to the medium.

RESULTS: All the materials were cytocompatible in terms of cell viability and metabolic activity for up to 7 days. The amount of mineral deposition as gene and protein expression analysis confirmed the ability of PLA functionalised with Gel and nHA to intrinsically stimulate the osteogenic commitment of Y201s. Comparing the pathological model to the healthy one at 21 days of culture, a higher expression of pro-angiogenic factor (vegf) and a decreased expression of osteogenic markers (coll1, spp1, runx2) in the SB, were detected. Moreover, the pathological model displayed a lower production of glycosaminoglycans, a higher content of collagen X, and a weakness in the mechanical performances in the AC deep layer.

DISCUSSION & CONCLUSIONS: This novel approach provides a new tool for studying the development and progression of OA characterized by quick manufacturing, easy manipulation and large availability.

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Keywords: Disease models, Microenvironment and niche engineering

OP-271

Impact of micro- and macro-mechanical environment in human cardiomyocyte biology using next-generation sequencing

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INTRODUCTION: The generation of human cardiomyocytes (CMs) derived from induced pluripotent stem cells (iPSCs) and tissue engineering have opened new ways for cardiac regeneration. However, the fabrication of truly mimetic cardiac tissues is limited by the lack of in-depth information on the effects of the bioartificial architecture and the mechanical environment imposed on the cells. Our work aims to use for the first time next-generation sequencing technology (Total RNA-seq-backed Spatial RNA-seq) in combination with human bioengineered myocardium to analyze the influence of the micro- and macro-mechanical environment on the evolution, maturation and mimicry of the biofabricated tissues, at unprecedented resolution.

METHODS: Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (hiPSC-CMs) and cardiac fibroblasts (hiPSC-CFs) were embedded in a fibrin hydrogel and combined with the 3D printed MEW-designs in PCL, forming a composite system. Cardiac engineered tissues were maintained in culture for up to 4 weeks and characterized by histology, staining, structure, plus electrophysiological and genomics analysis.

RESULTS: Contractile human cardiac minitissues can be generated using fibrin hydrogel reinforced with 3D MEW printed structures displaying metabolically active hiPSC-CMs, well distributed throughout the scaffold. Electrophysiological analysis showed that cardiac minitissues matured over time displaying calcium transient conduction velocities of up to 6 - 8 cm/s. A more specific structural examination determined that CMs followed and overall alignment dictated by the fibers of the MEW design. Genomics analysis with bulk RNAseq indicates an increased expression of genes related to cardiac maturity, lipid metabolism, and calcium metabolism in the 3D tissues compared to cells cultured in 2D. Interestingly, we detected an increased expression of extracellular matrix related genes in 2D cultures compared to 3D tissues demonstrating in agreement with previous studies, that 2D cultures do not reflect the in vivo reality. Finally, we analyzed the fiber-reinforced samples using

spatial RNAseq, of high relevance due to the different mechanical regions in the composite tissues (close to MEW fibers: stiff; within the hydrogel: compliant), providing novel information with an unprecedented precision.

DISCUSSION & CONCLUSIONS: Our MEW-based human cardiac engineered tissues display features of maturation at both structural and functional levels. The application of bulk- and spatial- RNAseq has provided a new mechanistic framework with which we are starting to understand how human cardiac cells are influenced by their local mechanical environment. This information will help us devise advanced tissues with better mimicry, increasing their therapeutic capacity.

Keywords: Biofabrication, Additive manufacturing

OP-272

Tracheal engineering to the reconstruction of the larynx

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INTRODUCTION: In France, each year, about 1,800 people undergo a total laryngectomy, which involves removing the entire larynx and placing instead a tracheostomy tube 1. However, the resulting tracheostomy orifice brings important difficulties.

An urgent demand encourages us to create an artificial larynx restoring sphincter function and extending the remaining trachea after total laryngectomy.

We previously designed a titanium artificial larynx and implanted it in 2012 2. However, this titanium artificial larynx has 2 main limitations 3: 1) a lack mechanical flexibility; and 2) limited tissue integration. As alternative, aortic allografts offer a promising approach in tracheal engineering 4.

To reconstruct artificial larynx, we carried out two strategies either 1) from aortic allografts or 2) from a totally biological cellularized tubular albumin scaffolds, using a bioreactor, to precondition them in vitro.

METHODS: We designed a rotating bioreactor for albumin scaffolds cellularization under dynamic perfusion. The bioreactor includes a double-chamber inside which the scaffold is held. Then, it is possible to co-culture two cell models (epithelial and chondrocyte cells with their own nutritional needs) on the endoluminal and external faces of the scaffold respectively.

RESULTS: Strategy 1: In vivo implantation of aortic allograft for laryngeal reconstruction

We implanted aortic allografts at the laryngeal site in 6 sheep. We observed a cellular colonization of the grafts with the appearance of neovascularization, suggesting a good cellular integration to the recipient tissues. A first patient was recently implanted with an aortic allograft for laryngeal reconstruction.

Strategy 2: Tracheal engineering of cellularized albumin scaffolds with co-culture using a bioreactor

Existing aortic allografts have 2 main limitations 4: 1) cost; and 2) organ donation. Thus, we created albumin scaffolds for in vitro and in vivo experimentations to regenerate a structure close to the larynx. We designed a bioreactor to precondition in vitro the scaffold. We seeded cells (epithelial and chondrocytes on both faces) onto the scaffold in order to accelerate its transformation into larynx tissue during implantation.

DISCUSSION & CONCLUSIONS: These tracheolaryngeal engineering studies led to the first clinical application of aortic allografts at the laryngeal site and opened the way to the use of albumin

materials in tissue regeneration

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Keywords: Biomaterials, Other

OP-273

Novel Hybrid Tissue Engineering Vascular Graft for surgical bypass grafting: hierarchical cellularization to mimic native arteries

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INTRODUCTION: Ischemic heart disease is the number one killer worldwide. Despite the improvements of the last decades, the goal of manufacturing a fully functional small diameter vascular conduit has not been achieved yet. Here, we aimed at developing a multi-layered hybrid TEVG combining the biological and physical characteristics of a living tissue.

This novel approach consists in a polymeric conduit, able to confer mechanical support, fully cellularized with specialized vascular cells in order to recreate the native multilayer structure of a native artery.

METHODS: The development of the hybrid TEVG was divided in two main steps: 1) polymeric conduit fabrication and 2) cellularization. 1) The polymeric core of the graft was manufactured with electrospinning technique (Fig. 1A). The inner surface of the conduit was made of a layer of gelatin (GL) 15%(w/v) nanofibers, crosslinked with γ -glycidipropyltrimethoxysilane (GPTMS), forming a nanometric network with high adhesive features for cells [1]. The outer surface was then fabricated with polycaprolactone (PCL) 15%(w/v) to confer mechanical strength and durability to the graft. Morphology and mechanical properties of the scaffolds were characterized through SEM and tensile testing. 2A) Lumen was seeded with endothelial cells (ECs) to create an anti-thrombogenic surface through an innovative rotating system and its parameters were optimized (Fig. 1B). 2B) The cellularization was completed with the formation of external cellular layers mimicking the structure of native artery.

The hybrid TEVG was tested in a swine model in which the graft was implanted as interposition of left pulmonary artery (LPA) for 5 weeks.

RESULTS: We demonstrated the success of the novel hybrid scaffold fabrication and robustness of assembling protocol (Fig. 1C). Mechanical properties showed values comparable with the human coronary artery (Young's modulus of 1.48MPa [2]). In vitro assays showed the achievement of a viable (above 90%) and uniform lining of ECs, fundamental as anti-thrombogenic property of the TEVG (Fig.1D). Histology demonstrated the accuracy of the cellularization methods.

At last, the TEVG was successfully implanted in a large animal model (Fig. 1E) demonstrating the therapeutic potential for vascular bypass surgery.

DISCUSSION & CONCLUSIONS: The novel hybrid TEVG resembling the structure of the native arteries represents an alternative in surgical revascularization with the potential to remodel and growth as a living tissue inside the host organism

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Keywords: Vascular systems / vascularisation and heart, Composite materials

OP-274

Evaluation of a tissue engineered nerve conduit for peripheral nerve regeneration of a rabbit fibular nerve defect

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INTRODUCTION: Even with all recent technologies, peripheral nerve repair remains a challenge. The gold standard technique, autologous nerve graft, induces a loss at the donor site while promoting a poor functional recovery in many cases¹. Nerve tube (NT) made of biomaterial can be used to guide axonal migration but faces several clinical limitations. In the nerve regeneration process, an adequate blood circulation is essential, but is not rapidly achieved with inert material. Our goal is to produce a viable NT using the patient's own cells and pre-seeded this NT with endothelial (ECs) to allow a faster recovery for patients. A nerve tube containing a pre-established capillary network, which can connect quickly to the host's microvasculature should be a promising option to repair large nerve defects (>3cm).

METHODS: NT are made of human fibroblast sheets seeded with ECs and rolled to form a tubular structure. NT were implanted in immunosuppressed New Zealand rabbit for 36 weeks to repair a 4 cm fibular nerve defect. Electrophysiological testing and functional assessment were performed. The nerve conduction at the fibular nerve was evaluated monthly under light anesthesia. The Toe Spread Index (TSI) was used to monitor the functional outcome².

RESULTS: The NT recovered significantly greater muscle mass than the negative control (unrepaired nerve). Nerve conduction analysis in the *tibialis anterior* muscle showed that nerve conduction recovery began around the 18th week. The electromyogram showed a resting electrical activity return with multiple polyphasic activities (i.e., reinnervation in progress) around the 24th week in autograft group. After 36 weeks, an improvement of the TSI showed a partial motor function recovery in rabbit with autograft ($p < 0.05$). A similar trend was also observed in the NT group.

DISCUSSION & CONCLUSIONS: Our team previously demonstrated in a rat model that pre-vascularized NT's allows for a better motor function recovery after a 15 mm nerve defect³. Our approach to develop a prevascularized living autologous NT promoting a rapid vascularization of the graft is essential to support axonal migration over long distances and could be a promising new clinical tool to repair large lesions (>3cm).

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Keywords: Nervous system (brain-central-peripheral / disorders), In vivo and animal models

OP-275

Injectable polyisocyanide-gel for pelvic floor surgery: connective tissue regeneration in an *in vivo* abdominal wound model

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INTRODUCTION: Pelvic organ prolapse (POP) is the descent of pelvic organs into the vagina when supportive tissues weaken [1]. In POP, vaginal fibroblast function is disturbed due to increased loading conditions (e.g. vaginal delivery), which alters collagen formation in connective tissue of the fascia [2]. The primary treatment option for POP is native tissue repair (NTR) which involves plication of healthy vaginal fascia to cover the fascial defect. However, the regeneration capacity of the connective tissue is low. We hypothesize that a gel functionalized with bioactive components may trigger fibroblast activity and connective tissue regeneration when applied during NTR. We modified polyisocyanide (PIC)-gels with basic fibroblastic growth factor (PIC-bFGF), estradiol (PIC-E2) and adipose-derived stem cells (PIC-ADSCs) and investigated tissue biomechanics and the host response in an abdominal wound model in a rabbit. The PIC-gel is a fully synthetic injectable, biomimetic gel that facilitates a regenerative response by vaginal fibroblast from POP tissue *in vitro* [3].

METHODS: In 20 New Zealand rabbits, abdominal wounds were created by 3 cm incisions. During wound closure, 0.5 ml PIC, PIC-bFGF, PIC-E2, PIC-ADSC or saline (sham) were blinded and randomly injected. Full-thickness tissues at the abdominal wounds were excised at 14 and 42 days post-surgery. Tissue biomechanics, by means of stiffness, was performed with uniaxial tensile testing. The host response was assessed by scoring the collagen formation (Masson's Trichrome), immune response (polymorphonuclear cells (PMNCs), foreign giant body cells (FGBCs); hematoxylin and eosin (HE)) and vascularization (HE).

RESULTS: The tissue stiffness and host response were constant between all groups within each time point. Over time, tissue stiffness only increased significantly when injected with PIC, PIC-E2 and PIC-ADSC ($p = 0.02$; $p = 0.001$ and $p = 0.005$, respectively). Regarding the host response, collagen formation only increased significantly in tissue injected with PIC, PIC-bFGF and PIC-ADSC ($p = 0.01$; $p = 0.03$ and $p = 0.03$, respectively). FGBCs, PMNCs and vascularization were mildly present and constant, with or without hydrogel.

DISCUSSION & CONCLUSIONS: The injection of abdominal wounds with PIC-gels resulted in increased collagen formation and tissue stiffness over time, whereas these outcomes did not change after sham-surgery. These findings indicate that injection with the PIC-gels potentially improves connective tissue regeneration. This study motivates us to investigate PIC-gel by vaginal injection in a NTR model in sheep.

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Keywords: Hydrogels and injectable systems, Biomaterials

OP-276

Tissue-Engineered Interfaces to Enhance the Biointegration of Neural Implants

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INTRODUCTION: Neural implants (NIs) enable bidirectional communication with the nervous system via electrodes, which record or stimulate the activity of neurons. Despite their widespread use in the clinic, surgical implantation invariably leads to local tissue damage and an inflammatory foreign body response (FBR) at the device-tissue interface. This results in physicochemical changes that progressively hinder implant performance and could lead to fibrotic encapsulation over chronic time scales [1]. In this regard, the stiffness mismatch between rigid arrays and soft neural tissues has been shown to be a key mediator of the inflammatory and fibrotic responses that underlie implant failure [2]. Therefore, biomaterial-based strategies hold great promise to engineer soft interfaces that enhance implant biointegration.

METHODS: We hypothesised that a tissue-engineering approach could be used to develop a biomimetic coating that recapitulates the properties and functionality of neural tissues. For this, a layered construct was engineered composed of a conductive hydrogel (CH) and an overlying carrier embedded with neural cells. The CH was synthesised based on the incorporation of poly(3,4-ethylenedioxythiophene) (PEDOT) into a taurine-modified poly(vinyl alcohol) (PVA) hydrogel. The hydrogel carrier was developed by combining norbornene-modified gelatin and PVA to produce a biosynthetic scaffold with tuneable physicochemical properties. The two hydrogels were then formed sequentially on platinum electrode substrates and the electrochemical properties of the assembly were assessed via impedance spectroscopy and cyclic voltammetry. Lastly, the ability of the engineered constructs to support the development of primary neural cells in vitro was evaluated via immunofluorescent staining.

RESULTS: Electrochemical characterisation showed that the CH improved the properties of platinum electrodes by providing a high charge transfer area with lower impedance and a more tissue-like structure. In addition, immunofluorescent staining showed that the hydrogel carrier could support the growth and differentiation of 3D encapsulated co-cultures of primary neural progenitors and astrocytes.

DISCUSSION & CONCLUSIONS: Tissue-engineered coatings were shown to improve the electrochemical properties of conventional metallic electrodes, while also providing a soft interface that mimics native neural tissues. It is hypothesised that these "living electrodes" could form functional connections with the host nervous system after implantation, thus establishing a seamless and chronically stable bioelectronic interface. In turn, this approach could lead to the development of safer and longer lasting NIs with improved clinical efficacy for different biomedical applications.

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Keywords: Biomaterials, Nervous system (brain-central-peripheral / disorders)

OP-277

Single-cell RNA sequencing of renal glomerular and tubular interstitial endothelium during vascular specific injury and regeneration

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INTRODUCTION: Previously, we demonstrated that endothelial repair in murine kidneys primarily depends on local endothelial specific mechanisms. Analyses of the marker genes, pathways, and biological functions revealed that endothelial cells (EC) are highly heterogeneous showing plasticity both in normal and pathophysiological conditions. However, the heterogeneity and cellular responses of renal ECs during injury and regeneration have not been well characterized

METHODS: Endothelial specific injury was induced in Tie2 eGFP reporter mice (n=48) by renal arterial perfusion with ConcanavalinA (ConA) /anti-ConA. Nineteen mice served as sham operated controls. Kidneys were harvested 24h, 48h, 3 days, 4 days, and 7 days after injury induction. For 10x single-cell RNA sequencing, more than 40,000 cells were isolated from glomerular and extraglomerular renal tissue. Tie2 EGFP+ CD102+ CD105+/CD45- endothelial cells were further separated using FACS cell sorting. After 10x single cell sequencing, Seurat clustering analysis was performed to identify EC cell clusters. Endothelial cell damage was evaluated using periodic acid–Schiff, acid fuchsin orange G and histology (CD31, ERG) in zinc fixed, paraffin embedded sections.

RESULTS: EC injury and loss was observed 24h following injury, while the EC number already markedly increased on day 3 and was back to baseline levels after 4–7 days. Following quality control measurements, almost all sequenced cells expressed endothelial specific genes. Especially the glomerular, cluster analysis revealed great genetic heterogeneity during injury (24-48h) and regeneration (day3-day7). Fourteen glomerular and seven peritubular EC clusters could be detected. Within the glomerular ECs, clusters with marker genes for capillaries (5 clusters), vas afferens, vas efferens, remodeling/differentiating (3 clusters) fibrotic/injury (2 clusters), activated capillaries and proliferation were identified. The peritubular EC clusters were associated with, capillaries (3 clusters), remodeling and regeneration, activated capillaries, arteries/arterioles and veins.

DISCUSSION & CONCLUSIONS: In summary, our study provides a high-resolution atlas of the renal endothelium during EC specific injury and regeneration. We highlight the phenotypic heterogeneity and changes of endothelial subtypes and gene patterns which are potentially involved in EC damage and repair in a highly specific murine injury model.

Keywords: Kidney, In vivo and animal models

OP-278

Supplementation of autologous fat grafts with lipoaspirate derived stem cells for the treatment of facial contour deformities

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INTRODUCTION: In reconstructive surgeries, fat grafting produces undesirable clinical outcomes due to high fat resorption and low graft survival rate at the site of application. In this context, adipose-derived stem cells (ASCs) exhibit promising potential to enhance the reliability of fat grafting. Despite ASCs supplementation, the pragmatic effects of ASCs augmented fat grafts are not perdurable over time due to hypoxic environment at grafting site. Curcumin a well known natural antioxidant, improves cellular viability and functionality. In the current study, curcumin preconditioned ASCs were used to induce cyto-protective signals in ASCs; thereby improving retention of fat and make more reliable therapeutic outcomes.

METHODS: In the current study, autologous lipoaspirate stem cells were cultured using GMP grade reagents and characterized for mesenchymal markers and tested for microbial and viral contamination. ASCs primed with 5µM curcumin for 24hrs were used to induce cytoprotective signals in ASCs; thereby improving retention of fat and to make more reliable therapeutic outcomes. This study was proposed as randomized controlled clinical trial with three parallel interventional arms; conventional fat grafting, cultured ASCs enriched fat grafting, and, curcumin-preconditioned ASCs enriched fat grafting. The trial is registered with NCT05610878. In all groups, adipose tissue was injected at the site of facial atrophy till visual clinical symmetry with the opposite side was achieved. In total seven patients which included three patients (Perry Romberg syndrome) with only fat grafting, three patients of ASCs enriched fat grafting (with one each of Perry Romberg syndrome, Hemifacial microsomia, and accidental scar), and one patient with curcumin primed ASCs enriched fat grafting have been included. The subcutaneous thickness of injected fat graft was measured after one week, 3, 6 and 9 months using a B-mode ultrasound device connected with a 12 MH frequency probe. The decline in the volume of transplanted fat, surgeon assessment scores based on interview and visual inspection of fat volume, and patient self-assessment scores based on texture, softness, itching, pain, as well as contour were also recorded.

RESULTS: After 9 months, there was lower fat resorption, significant improvement in surgeon and patient self-assessment scores in the curcumin-preconditioned ASCs enriched fat grafting group as compared to other groups. After transplantation, no major donor site complications were noted in any patient.

DISCUSSION & CONCLUSIONS: The results suggest that preconditioned ASCs supplemented fat graft procedure is effective and safe for soft tissue restoration in facial contour deformities and may be superior to other therapies.

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Keywords: Stem cells - general, Wound healing

OP-279

Cyclic Straining of In Vitro Tissue-Engineered 3D Models: Investigating the Effect on the Extracellular Matrix

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INTRODUCTION: As cells and tissues in our body are continuously exposed to mechanical forces, mechanical loading should be incorporated in in vitro 3D models. Polyisocyanide (PIC) hydrogels are synthetic, thermosensitive and highly biomimetic soft materials that display many of the characteristics of biological matrices [1]. Therefore, the PIC hydrogel has great potential to mimic the 3D native extracellular matrix (ECM) and is an ideal substrate for investigating the effect of mechanical loading of tissue-engineered constructs. In this study, we investigated the effect of cyclic straining on 3D-cultured human adipose-derived stem cells (ADSCs) focusing on ADSC differentiation, gene and protein expression of ECM proteins and collagen alignment.

METHODS: PIC polymers were synthesized and conjugated with a cell-adhesive peptide GRGDS as previously reported [2]. ADSCs (passage 5) were encapsulated in the PIC hydrogel. Eight days post-encapsulation, cyclic straining (2%, 0.2 Hz) was applied for 7 days using the Flexcell® FX-5000TM Tension System. The Flexcell® Bioflex plate was adjusted using similar modifications described in Wissing et al. [3]. The static controls did not receive any mechanical loading. The effect of cyclic straining, versus static controls, was evaluated for gene expression of ECM production and remodeling related genes, collagen alignment and deposition (protein visualization with CNA-OG488), and elastin deposition.

RESULTS: The 3D cultured ADSCs can be successfully cultured up to 7 days of cyclic straining. Cyclic straining highly promotes collagen production. The dynamic samples show an anisotropic collagen fiber alignment, parallel to the strain direction, which is absent in the static control samples. Cyclic straining significantly upregulates the expression of alpha smooth muscle actin ($p = 0.0017$), collagen type III ($p = 0.0115$) and elastin ($p < 0.0001$).

DISCUSSION & CONCLUSIONS: Cyclic straining results in increased myofibroblast differentiation, collagen production and alignment of tissue-engineered constructs, confirming that the PIC hydrogel transfers the mechanical loading to which the cells respond. In the future, we would like to apply our setup to examine the effect of mechanical loading in the context of pelvic organ prolapse (POP). Hereby, we would like to assess whether POP fibroblasts respond differently in terms of collagen metabolism compared to non-POP fibroblasts. Moreover, incorporating mechanical loading in complex 3D in vitro models can result in more translatable disease models in the future.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Hydrogels and injectable systems

OP-280

Cell's Sense of Slope

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INTRODUCTION: Signals from the extracellular matrix influence spatio-temporal organization of cells and tissues, guiding many processes such as morphogenesis, pathology, and repair. A physical cue that is gaining importance in this field is the role of local geometry. Here, the effects of surface curvature on the focal adhesions (FAs) dynamics and cytoskeletal assembly have been investigated by using a molecular-mechanical model. The model outcomes have been validated exploring cells mechanics on curved substrates.

METHODS: Cells probe tissue properties through their adhesive molecular machinery that is referred to as a “molecular clutch”. We developed a modified version of the molecular clutch model [1], to explain cell behaviour in response to concave and convex substrates. Master equations governing the dynamic evolution of the system take in consideration the spatial organization of the actin stress fibers according to the slope of the substrate and Monte Carlo simulations have been used to describe the clutch binding and unbinding events. Single cell analyses have been performed to characterize the mechanical identity of NIH/3T3 seeded on curved PDMS substrates. The substrates consist of microarray of concavities/convexities with controlled radii of curvature.

RESULTS: On concave surfaces, the cell reaches its equilibrium condition with a lower number of clutches bounded respect to the planar case. Specifically, it decreases as the concavity of the substrate increases. Contrariwise, the simulations on convex substrates identify a trend that is completely reversed, in fact, a higher number of clutches is involved in the cell adhesion process. In addition, the model predicts that these substrate slope-dependent changes in clutch dynamics lead to substantial differences in the traction forces exerted on curved versus flat substrates. Cells exert a force that increases as the convexity of the substrate increases. We confirmed model predictions experimentally. Focal adhesion size was found to increase shifting from concave to convex substrates. The same trend was verified in terms of cell Young's modulus using atomic force microscopy.

DISCUSSION & CONCLUSIONS: The concavity induces a softening effect in the cellular perception of substrates with cells adopting a spider-like configuration characterized by smaller FAs and a lower mechanical integrity than in the planar case. On the other hand, the convexity induces a stiffening effect that results in cells adopting a snail-like configuration with larger FAs and a more robust cytoskeleton assembly. Cell mechanosensing is not mediated only by substrate stiffness but also the curvature plays a key role in the mechanical response of the cell.

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Keywords: Microenvironment and niche engineering, Biomaterials

OP-281

Mammary epithelial organoids cultured in a self-assembling peptide hydrogel exhibit stiffness-induced remodelling

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INTRODUCTION: Breast tissue stiffness is known to play a role in driving breast cancer development and progression via mechano-sensitive signalling pathways, but the specific mechano-signalling events involved are poorly understood. Three-dimensional (3D) organoid models can assist with the identification of molecular mechanisms that promote these phenomena. Matrigel is a mouse-derived hydrogel composed of extracellular matrix (ECM) proteins and growth factors that can support organoid culture. However, Matrigel has many limitations, and there remains a need for tuneable, synthetic model systems that can reproducibly replicate the biochemical and mechanical properties of the mammary ECM. Here we demonstrate that the peptide hydrogel PeptiGel Alpha4 can support culture of human mammary epithelial cells (MECs), and that the mechanical properties of the hydrogel can be tuned to induce mechano-sensitive cellular behaviours.

METHODS: Immortalised, non-tumorigenic human MECs (MCF10a, ATCC) were encapsulated in Matrigel-alginate (Corning; Novamatrix) or PeptiGel Alpha4 (Manchester BioGEL) hydrogels. The stiffness of hydrogels was tuned by dilution; elasticity was measured by oscillatory shear rheometry (Discovery HR-2 Hybrid Rheometer, TA Instruments). Organoids were characterised by light and confocal immunofluorescence microscopy (Leica TCS SP8 AOBS upright confocal using a 63x/0.90 water immersion objective), with staining against caspase-3 (R&D Systems AF835), laminin alpha-3 chain (R&D Systems MAB21441), collagen-IV (Abcam ab6586), and beta-catenin (BD Biosciences 610154). The cell-derived matrisome was analysed by liquid chromatography coupled tandem mass spectrometry (LC-MS/MS; QExactive HF, Thermo Scientific) and MaxQuant software.

RESULTS: Dilution of the PeptiGel Alpha4 hydrogel allowed its stiffness to be tuned between 400 and 150 Pa (100% to 50% volume/volume dilution). The synthetic peptide hydrogel was able to support human MEC viability and the formation of organoids over a period of three weeks. We also showed that cell-derived ECM proteins could be extracted from the hydrogel culture systems and analysed using mass spectrometry. MECs encapsulated in the Alpha4 hydrogel were found to synthesise basement membrane proteins, such as laminins. Increased Alpha4 matrix stiffness was found to reduce MEC viability, alter matrix production and cause an increase to inflammatory, immune and antioxidant responses.

DISCUSSION & CONCLUSIONS: Our findings demonstrate the potential of PeptiGel Alpha4 to build in vitro model systems with tuneable mechanical properties, thus enabling examination of pro-oncogenic mechano-signalling events.

Keywords: Hydrogels and injectable systems, Cancer

OP-282

The Role of GPR-161 in Osteocyte Mechanotransduction and Osteoclastogenic Paracrine Signalling

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INTRODUCTION: Osteocytes comprise 90-95% of cells within bone which form a sensory network throughout the tissue orchestrating bone remodelling and adaptation to physical loading [1]. However, the specific molecular components of osteocyte mechanotransduction remain poorly understood [2]. GPR-161 is a G-Protein coupled receptor that has recently been identified to be involved in MSC mechanotransduction, but whether GPR-161 is expressed or utilised by osteocytes is unknown [3]. Therefore, this study aimed to investigate the role of GPR-161 in osteocyte mechanotransduction and subsequent osteoclastogenic paracrine signalling.

METHODS: GPR-161 expression in MLO-Y4's was verified at a mRNA and protein level with RT-qPCR and immunocytochemistry (ICC) respectively. Mechanical stimulation was applied utilising parallel flow chambers to provide oscillatory fluid shear (OFS) at 1Pa, 1Hz for 2 hours. Knockdown (KD) of GPR161 was achieved via transfection of siRNA via lipofectamine. CD14+ human blood-derived monocytes were plated in growth media with M-CSF and RANKL supplementation in an established osteoclastogenesis assay, which was treated 1:1 with stimulated osteocyte conditioned media (CM). Cells were stained for TRAP activity according to the manufacturer's protocol followed by DAPI staining to determine osteoclast number.

RESULTS: GPR-161 is expressed in MLO-Y4's at both an mRNA and protein level. ICC staining indicates a pattern of colocalisation at sites of focal adhesions. Following exposure to OFS osteocytes increase expression of COX2 (2.1-fold) and GPR-161 (1.4-fold) and decrease RANKL (0.4-fold). Upon KD of GPR-161 in MLO-Y4 cells, RANKL expression is significantly decreased compared to the scrambled control. Moreover, RANKL expression is not affected by OFS following GPR161 KD.

CM from mechanically stimulated MLO-Y4's significantly reduced osteoclastogenesis in contrast to CM from statically cultured osteocytes. Following GPR-161 KD in MLO-Y4's, a significant drop in osteoclastogenesis was observed in the static group due to a decrease in monocyte viability. However, in mechanically stimulated MLO-Y4 cells lacking GPR-161, CM did not influence osteoclastogenesis when compared to the treatment control.

DISCUSSION & CONCLUSIONS: GPR-161 was shown to be expressed in osteocytes and there is colocalisation of GPR-161 at focal adhesions. This presence at known mechanosensitive sites could be indicative of a role in osteocyte mechanobiology. Without the expression of GPR-161, osteocyte secreted factors increased osteoclastogenesis despite being exposed to OFS, differing from scrambled control groups. These results indicate GPR-161 plays a role in osteocyte mechanosignalling and osteoclastogenic paracrine signalling.

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Keywords: Bone and bone disorders (osteoporosis etc), Bioreactors

OP-283

Closed-loop biaxial cell stretching system for controlling cell mechano-transduction processes

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INTRODUCTION: Deformable cell culture systems, such as cell stretcher device, proved to modulate cell behaviour through a fine tuning of mechano-sensing/transduction events occurring at cell-material interface. Although a heterogeneous category of cell stretchers offers high performances and flexibility in modulating cell mechanics, nowadays a precise automatized cell stretching system capable to operate in a closed-loop system along with cell biochemical and biophysical response is still missing. Along these lines, this work presents the development of a custom-made biaxial cell stretcher equipped with a feedback loop on nucleus morphology for the control of cell mechano-transduction processes.

METHODS: The custom-made biaxial stretching device is mounted on top of an inverted fluorescence microscope allowing the stretching of a PDMS cell chamber and live cell fluorescent imaging. A nanopatterned (ridge and groove width of 700 nm and ridge depth of 350 nm) PDMS membrane is present at cell chamber's bottom to align cells in a preferential direction and obtain a uniform cell response and nuclear deformation upon substrate stretching. A proportional-integral MATLAB control algorithm is responsible to interface the microscope proprietary software and the actuation system, creating a closed-loop system with feedback control of nuclear morphology. In particular, once provided the desired nuclear major and minor axis strain, the entity of substrate stretch is automatically and dynamically adapted by the control algorithm until desired configuration is reached.

RESULTS: The orientation of more than 75% of NIH 3T3 cell number on membrane's nanopattern falls within the $\pm 10^\circ$ range respect to the nanopattern direction. From preliminary cell stretching experiment, in just three iterations, the control algorithm is able to bring the nuclear axes strains to the 10% error range of the reference through subsequent biaxial stretch of the substrate. Moreover, as soon as the nuclei begin to retreat and the control variable exits the reference range, the control algorithm automatically and dynamically changes the entity of membrane stretch in order to restore and maintain the desired nuclear deformation.

DISCUSSION & CONCLUSIONS: This work presents a first example of synergy between Mechanobiology and Automation Engineering. Next steps will be the introduction of biochemical information (gene/transcription factor localization and expression) in the closed-loop system in order to explore the possibility of monitoring and controlling also the epigenetic effects associated to the nuclear deformation induced by cell stretching. We expect to elucidate the mechanisms behind the mechano-transduction processes and give important insights about the mechanical dosing necessary to guide certain cell functions or fate.

Keywords: Biomaterials,

OP-284

Effects of different mechanical stimuli on engineered cardiac tissues

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INTRODUCTION: The establishment of functional three-dimensional engineered cardiac tissue (ECT) models is crucial for effective drug screening and deepen the understanding of cardiac development and regeneration mechanisms. It is known that the application of physiological cues mimicking those typical of the native myocardium promotes the cardiac maturation and functionality *in vitro*¹. The limit of the commercially available bioreactors is the application of one-two physical force type at a time often with a limited loading range². To overcome these limitations, a millimetric-scale microscope-integrated bioreactor was developed to deliver simultaneously multiple biophysical stimuli, namely passive, active loadings and electrical stimulation, to ECTs³. In this study, the effects of the application of a single passive stimulation or in combination with an active mechanical stimulation were investigated.

METHODS: Hydrogel-based ECTs were generated by seeding rat origin cardiac cells (70%Cardiomyocytes + 30%Fibroblasts) in a 100 μ L fibrin gel solution (25 mg/mL fibrinogen, 5 U/mL thrombin) at the density of 20×10^6 cells/mL. The fibrin gel-based constructs were culture for 8 days in passive stimulation and in static condition as control. A further mechanical loading regime combining passive (8 days) and active (10% deformation at 1Hz for the last 4 days of culture) stimulations was investigated. The functionality of ECT was assessed by measuring the Excitation Threshold (ET), as the minimum electrical field at which ECT starts beating synchronously, and the Maximum Capture Rate (MCR) as the maximum frequency that ECT can follow. Cardiac maturation was assessed by immunofluorescence for specific cardiac markers.

RESULTS: ECT exposed to passive stimulation showed superior functionality compared to static cultured constructs. In particular, the ET of passive stimulated ECT was almost 10-fold lower than the static condition. Immunofluorescence for a specific cardiac marker (sarcomeric α -actinin) showed a superior sarcomere organization, cell elongation in ECT passively stimulated compared to controls. Preliminary results showed that the sequential application of active stimulation upon passive stimulation further enhanced the functionality (in terms of $ET=3.31 \pm 1.83V/cm$ and $MCR=4.00 \pm 0.76Hz$), developed force ($0.22 \pm 0.25mN$), and cell elongation within the ECT, compared to passive loading alone ($ET=8.11 \pm 5.87V/cm$, $MCR=3.00 \pm 1.07Hz$, and developed force= $0.04 \pm 0.03mN$).

DISCUSSION & CONCLUSIONS: The different mechanical loading types (passive followed by active loading regimes) applied during *in vitro* culture appear to improve the functionality of ECTs, leading to *in vitro* cardiac models of superior quality in terms of functionality and maturation level.

ACKNOWLEDGEMENTS: Swiss National Science Foundation (310030_172989 to A.M.).

REFERENCES: ¹Goldfracht, I. et al. (2019), ²Mittal, R., et al. (2019), ³Pisanu, A. et al. (2022)

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

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A cell compression tool to assess the response of tumor spheroids to compression in bioprinted hydrogels

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INTRODUCTION: Cell responses to mechanostimulation are in part dependent on the extracellular matrix's (ECM) mechanical properties as well as cell:ECM interactions. Mechanoresponses are triggered through activation of mechanosensitive ion channels such as Piezo1, which gates the influx of extracellular calcium. In cancer, the ECM surrounding growing tumors represents a compressive force and typically stiffens due to remodelling by tumor and stromal cells. During metastasis, the behaviour of cancer cells is affected by the mechanical properties of the different ECM they migrate to. The ECM may differ greatly between the primary and secondary tumor sites; as for example breast cancer cells metastasise to bone. For the MDA-MB-231 breast cancer cell line, we established that compression-induced Piezo1 activation alters their capacity to engage in behavior required for certain modes of migration in 2D [1]. Here we aim to explore these mechanoresponses in tumor spheroids enclosed in 3D hydrogel environments that represent breast and bone ECM, to better model conditions expected in vivo.

METHODS: In the current study, tumor spheroid mechanoresponses are triggered by compression using the Cell press tool, which consists of a silicon pillar, actuated along a piezoelectric track, which allows for controlled compression of spheroids with μm steps. The pillar is mounted in a donut load cell, permitting dynamic measurements of compression forces while simultaneously allowing live imaging of the spheroids. The Cell press device has previously been applied to study Piezo1 activity in MDA-MB-231 cells [1], and mechanosensitivity in organoids (pancreatic islets) [2]. To assess spheroid mechanosensitivity, changes in cytosolic calcium are monitored by live imaging of spheroids labelled with Fluo4 and Fura red fluorescent calcium reporters during different degrees of compression and in different bioprinted hydrogels.

RESULTS: Here we demonstrate the capacity of the Cell press to compress and sense changes in force over a range of piezoelectric step sizes. We have generated tumor spheroids with an average diameter of approximately 500 μm and imaged changes in cytosolic calcium before and after Cell press-mediated compression. We are currently characterising the mechanoresponses of spheroids in a hydrogel-free setting; in spheroids embedded in hydrogels; and in bioprinted hydrogels containing spheroids.

DISCUSSION & CONCLUSIONS: These models will be used to elucidate the impact of hydrogel stiffness and composition on the mechanosensitivity of tumor cells in different 3D microenvironments.

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[2] Sanchez et al. 2022, J. Cell Biol

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Cancer

OP-286

Development of an *ex vivo* liver perfusion model for research purposes

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INTRODUCTION: Normothermic *ex vivo* organ perfusion enables the preservation of an organ in a physiological state. Although mainly introduced for organ transplantation, such technique showed also useful results in pre-clinical research, potentially bridging the gap between *in vitro* models and *in vivo* clinical effects. In this study we used porcine slaughterhouse procured livers in an *ad hoc ex vivo* normothermic perfusion platform and aimed to investigate the applicability of our model for disease modelling and cell therapy which is in compliance with the 3R principles.

METHODS: Procurement and treatment protocol of livers in the slaughterhouse were developed and conducted. Livers were first prepared by cannulating vein, artery, and bile duct, and subsequently attached to the extracorporeal perfusion circuit and reperfused. Physiological pressures of 10 mmHg and 80 mmHg for portal vein and hepatic artery were applied, respectively. Perfusate (whole blood) was heated to 39°C. Hemodynamic parameters and blood gases were monitored. Liver viability and functionality was assessed through bile production, indocyanine green clearance, blood chemical analysis and tissue histology.

RESULTS: Applied pressure resulted in physiological flowrates during perfusion for all livers. The perfused livers remained viable and functional up to 12 hours. Investigation of the effect of different blood additives, such as amino acids, bile acids or immune suppressors showed an improvement of liver viability. Further, to study liver disorders the addition of free fatty acids and acetaminophen during liver perfusion resulted in early biochemical signs of liver damage, including reduced functionality. Additionally, first trials of spheroid infusion showed the suitability of the system as a potential alternative model for cell therapy research.

DISCUSSION & CONCLUSIONS: In conclusion, slaughterhouse livers were kept viable and functional for up to 12 hours. Perfusion time could not be extended further due to hemolysis formation after 12 hours. Nevertheless, *ex vivo* liver perfusion using slaughterhouse procured livers was shown to be an accessible platform to perform interventional liver studies in a functional, relevant large animal model while avoiding using laboratory animals. With further improvements to the model, *ex vivo* liver perfusion using slaughterhouse procured livers might become a versatile tool for studying liver diseases as well as liver cell therapy.

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Keywords: Cell therapy, Disease models

OP-287

Introducing osteoclasts in *in vitro* models of marrow stromal cells-derived mineralised cartilage in the context of endochondral ossification

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INTRODUCTION: The process of cartilage and early bone resorption during endochondral ossification is a crucial step for the remodelling of the tissue and formation of a mature bony structure. To date modelling endochondral ossification *in vitro* is limited to the initial steps of the process, namely induction of chondrogenesis and mineralisation of the newly formed matrix. In this work we introduced a new level of complexity in the *in vitro* modelling of endochondral ossification through co-culture of transient cartilage undergoing mineralisation and monocyte-derived osteoclasts. With this model we aimed to investigate how chondrogenic and mineralised pellets influence osteoclast formation.

METHODS: Chondrogenic and mineralised pellets were formed from paediatric human marrow stromal cells (hMSCs) cultured up to 21 days in the presence of transforming growth factor beta-3 (TGF- β 3) (chondrogenic) or TGF- β 3 and β -glycerophosphate (mineralised). Indirect and direct co-culture systems were performed by co-culturing pellets with CD14+ human monocytes or mature osteoclasts in a consensus medium (α MEM 5% FCSi, ascorbic acid, RANKL and M-CSF), within a transwell or seeding monocytes directly on the pellet surface, respectively. Osteoclastogenesis was evaluated by tartrate-resistant acid phosphatase (TRAP) staining and gene expression analysis (*DC-STAMP*, *OC-STAMP*, *CTSK*, *TRAP* and *ATP6V0D2*). Chondrogenesis was assessed by thionine, and collagen type II/X staining, as well as gene expression analysis (*COL II* and *COL X*). Mineralisation was determined by von Kossa staining.

RESULTS: We achieved osteoclastogenesis in the presence of chondrogenic or mineralised pellets, as demonstrated by positive TRAP staining of multinucleated cells and induction of osteoclastogenic markers. Interestingly, we found that chondrogenic pellets cultured with TGF- β 3 and dexamethasone until the start of the co-culture prevented osteoclastogenesis of monocytes, and that these factors must be removed at least 3 days before co-culture. This is not necessary in the case of pre-differentiated osteoclasts, since they did not witness such detrimental effect. Upon co-culture, chondrogenic and mineralised pellets underwent structural changes, consisting in a reduction in glycosaminoglycan amount and collagen type II and X. Finally, we successfully set-up a direct co-culture system of monocytes and chondrogenic pellets, and showed that monocytes can not only attach to the pellets but also differentiate *in situ* into TRAP-expressing cells.

DISCUSSION & CONCLUSIONS: This study demonstrates that osteoclasts can be generated in the presence of chondrogenic and mineralised pellets, and interactions between them can be studied. Further development of this model will provide us with a tool to study endochondral bone formation and ultimately resorption of mineralised cartilage *in vitro*.

ACKNOWLEDGEMENTS: Grant EMCLSH21004, Health~Holland.

Keywords: Bone and bone disorders (osteoporosis etc), Multipotent (mesenchymal) stem cells

OP-288

Multiphysics three-dimensional model of a bioengineered haematopoietic platform for platelet production

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INTRODUCTION: Developing a practical strategy to produce platelets in vitro exhibits a growing interest in transfusion medicine and tissue engineering. In nature, platelets are made in the bone marrow haematopoietic niche by megakaryocytes, cells that protrude cytoplasmatic extensions into the lumen of bone marrow sinusoid capillaries. Here, the shear stresses generated by the blood flow allow the detachment of platelets which enter circulation [1]. Thus, a bioengineered haematopoietic model needs to mimic the 3D architecture, composition, and hydrodynamic environment of the native tissue.

METHODS: Our group used silk fibroin to fabricate a 3D porous scaffold that promotes platelet production by differentiated megakaryocytes in vitro [2]. The silk scaffold was implemented into the perfusion chamber of a milli-fluidic optically accessible bioreactor (MOAB) [3]. The perfusion rate of the bioreactor was predicted via a computational finite element multi-physics model carried out in a COMSOL® multi-physics platform, where the scaffold geometry was obtained with high-resolution images acquired using an X-ray micro-tomography scanner. Platelet production was quantified and analysed in terms of morphology and functionality. Additionally, megakaryocyte metabolism was evaluated during the platelet release process via FLIM analysis.

RESULTS: Thanks to the combination of interstitial perfusion and confocal optical accessibility, the bioreactor allowed complete control of the local conditions imposed on cells in terms of fluid dynamics and mass transport. Flow rates lower than 600 $\mu\text{L}/\text{min}$ guarantee a shear stress distribution inside the culture chamber within the physiological range of 0-410 mPa [4][5]. Further, the pO_2 predicted, ranges from 14 to 20% consistently with blood oxygenation in capillaries. Interestingly, real-time analysis of the metabolic profile showed that cells mainly rely on glycolysis at high shear stress, thus suggesting that fluid flow may influence cell function in an unexpected way.

DISCUSSION & CONCLUSIONS: Thus, the finite element model successfully predicted the distribution of oxygen gradient inside the bioreactor guaranteeing normoxic culture conditions and the shear stress at the fluid-cells interface for several values of perfusion flow rates. Our bioengineered haematopoietic niche, coupled with the computational model for prediction/monitoring/control, represents a promising candidate for producing platelets since the main physical, biochemical, and metabolic features of the platelet production process can be reproduced.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In silico models

OP-289

Protease-sensitive alginate hydrogel-based 3D matrices via SPAAC chemistry

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INTRODUCTION: Alginate is a natural polymer widely used to create artificial extracellular matrices (ECM) for tissue engineering applications. Rendering alginate hydrogels susceptible to cell-driven enzymatic remodelling leverages their properties as ECM mimics but requires effective chemical strategies[1,2]. Herein, we explored the biocompatible/bioorthogonal strain-promoted azide-alkyne cycloaddition (SPAAC) to graft bi-functional matrix metalloproteinase (MMP)-sensitive peptides to cyclooctyne-modified alginate (ALGK) for partial crosslinking, followed by clicking integrin-binding peptides. Multifunctional hydrogels were formed via a secondary ionic-crosslinking step. Hydrogel properties were fine-tuned to provide cell-responsive/instructive 3D microenvironments.

METHODS: Ultra-pure alginate (ALG) was modified by carbodiimide chemistry with BCN-amine (K) to incorporate SPAAC-clickable cyclooctyne moieties (ALGK)[3]. Modification degree (MD) was assessed by ¹H-NMR. MMP-sensitive GGYGPVGLIGGK (PVGLIG) and GGGGRGDSP (RGD) peptides were modified with azides(N₃) and analyzed by HPLC-MS/MS. Bi-functional N₃-PVGLIG-N₃ was SPAAC-clicked to ALGK at different concentrations (0-250μM) and RGD-N₃ at 800μM. Peptide incorporation was analyzed by UV/VIS spectroscopy, Triple-SEC, FTIR, rheometry, and SPAAC-clickable fluorescent-tags. Multifunctional PVGLIG/RGD-ALGK gel-precursor mixtures were ionically crosslinked with Ca²⁺. Hydrogels' susceptibility to enzymatic-cleavage by collagenase was assessed. 3D-cultured fibroblasts (hDNF) were analyzed for viability (live-dead assay), metabolic activity (resazurin), gene expression (qPCR), MMP-production (zymography, immunostaining), morphology, and ECM production (confocal microscopy).

RESULTS: ¹H-NMR confirmed successful cyclooctyne incorporation in alginate (new peaks: 2.18-2.35/3.34ppm, MD≈3%). HPLC-MS/MS proved effective N₃-modification of both peptides by mass/charge ratio increase. PVGLIG-ALGK grafting was confirmed by concentration-dependent increase of UV/VIS-absorbance, and appearance of characteristic FTIR-peaks along with higher molecular-weight species. Hydrogels with 125μM PVGLIG presented a good compromise between the amount of incorporated PVGLIG and the double-end grafting percentage, which promotes effective crosslinking. The corresponding hydrogels presented higher stiffness, likely due to PVGLIG-mediated crosslinking. When used for 3D-culture of human fibroblasts (hDNF), these hydrogels supported extensive cell spreading and endogenous ECM deposition. The 3D-cultured hDNF expressed PVGLIG-cleaving enzymes, namely MMP-2 and MMP-14, that are central for ECM remodeling, and their secretome cleaved PVGLIG moieties.

DISCUSSION & CONCLUSIONS: We successfully produced protease-sensitive alginate hydrogels by click-chemistry followed by ionic crosslinking. Double-end PVGLIG grafting provided partial crosslinking of the hydrogel network, which was sensitive to cell-driven enzymatic cleavage. This translated into higher hydrogel stiffness but also higher cell spreading via hydrogel proteolytic remodelling. Collectively, we show that SPAAC enables the creation of biologically-relevant 3D microenvironments based on multifunctional alginate hydrogels.

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Keywords: Hydrogels and injectable systems, In vitro microenvironments

OP-290

Training-on-a-Chip: a multi-organ device to study the effect of muscle exercise on insulin secretion "in vitro"

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INTRODUCTION: Organ-on-a-chip (OOC) devices offer new disease modelling and drug discovery approaches by providing biologically relevant models of tissues and organs in vitro integrated with biosensors. Miniaturized biosensors systems and advanced tissue fabrication procedures enabled researchers to create multiple tissues on a chip with a high degree of control over experimental variables for high-content screening applications. In this work, we have fabricated a biomimetic multi-OOC integrated device composed of skeletal muscle and pancreatic cells to study the impact of exercise on insulin secretion [1].

METHODS: We developed a microfluidic device to accommodate, link, and analyze the cross-talk between the two biological models. This device consists of a PDMS microfluidic system with two open chambers to integrate the biological samples and a PMMA enclosure that seals the fluidic system and electrodes to apply different stimulation regimes to the skeletal muscle. Skeletal muscle was engineered using myoblast cells encapsulated in a matrigel-fibrinogen scaffold on a PDMS platform with flexible pillars. Pancreatic beta-cells were laden in a highly porous scaffold forming pancreatic pseudoislets. Finally, optical biosensors (localized surface plasmon resonance, LSPR) were fabricated using metaplasmonic materials and integrated in situ with the microfluidic device.

RESULTS: Engineered tissues have benefited from novel scaffolds and have been integrated with bioreactors, an electrical stimulator, and biosensors to monitor myokine secretion from skeletal muscle cells, insulin production, and effects of skeletal muscle contraction on beta-cells. The multiplexing quantification with high sensitivity (limits of detection of 0.01 and 0.02 ng/mL for IL-6 and Insulin, respectively) of secreted proteins due to the tissue cross-talk is a unique and novel feature presented in this work.

DISCUSSION & CONCLUSIONS: We have fabricated a biomimetic multi-OOC integrated device composed of skeletal muscle and pancreatic cells to study the impact of exercise on insulin secretion. Both engineered tissues are integrated with optical biosensing technology to monitor in real-time the contraction-induced myokine secretion and its effect on beta-cells insulin production. Using this device, we have demonstrated that insulin secretion is induced directly by the effect of myotubes contractions. Our cross-talk platform would improve drug test approaches and provide a new model to study the loss of pancreas functionality associated with T2D.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Enabling technologies

OP-291

Engineering of functionally active human myotubes and myobundles *in vitro*

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INTRODUCTION: Typ-2-Diabetes (T2D) is a pandemic disease affecting 450 million subjects worldwide. Regular performed exercise improves insulin sensitivity and glucose tolerance, markedly reducing the risk for T2D. The most utilized organ during exercise, skeletal muscle, responsible for 85% of insulin-dependent glucose uptake, releases myometabokines that systemically affect organs relevant for glycemic control like liver, adipose tissue and brain. Current *in vitro* differentiation protocols for modelling skeletal muscle tissue are incapable of fully recapitulating its physiological functions especially regarding exercise.

Aim of this study was to utilize IGF-1 to engineer a more physiological myotube and myobundle model from primary human skeletal muscle satellite cells *in vitro*.

METHODS: Primary human CD56-positive myoblasts were enriched using MACS applied to satellite cells obtained by percutaneous needle biopsies performed on vastus lateralis muscle. Expanded myoblasts were differentiated into myotubes in α -MEM, supplemented with palmitate, oleate and carnitine in the presence or absence of IGF-1 over the course of 10 days. Daily samples were analyzed by proteomics, qRT-PCR and treated with electrical pulse stimulation (EPS) to characterize contractibility. Respiratory measurements by seahorse, western-blot analyses and glucose-uptake measurements were performed. In addition, myoblasts were cultured in collagen-hydrogel-scaffolds and differentiated for 8 days to form functionally active myobundles with linear myotube alignment.

RESULTS: Differentiation into myotubes in the presence of IGF-1 led to the formation of thicker multinucleated myotubes in monolayer that showed physiological contraction upon EPS following day 6 while in the absence of IGF-1, obtained myotubes were incapable of contraction. Proteomic analysis showed upregulation of proteins in the presence of IGF-1 that contribute to striated-muscle development/contraction, myofibril- and sarcomere-assembly as well as subunits of the mitochondrial respiratory chain. Elevated expression of PGC1 α , MYH7 and reduced MYH1/2 suggested a switch towards slow oxidative fibers in line with elevated mitochondrial respiration capacity. Upregulated expression of GLUT4, retained inducibility of the insulin-signaling cascade and increased insulin-dependent glucose uptake, after 48h of IGF-1 fasting, hinted towards an advanced model, better recapitulating physiological traits of skeletal muscle *in vivo*. Culture in a collagen-hydrogel-scaffold with this novel differentiation protocol further allowed engineering of linearly aligned contracting myobundles.

DISCUSSION & CONCLUSIONS: In conclusion, by utilizing IGF-1 and a collagen-hydrogel-scaffold we engineered human myotubes and 3D-myobundles from primary human myoblasts that recapitulate the physiological traits of skeletal muscle *in vivo* vastly superior to established protocols. This model

can be used to investigate exercise on a molecular level, screen drug effects and investigate interorgan crosstalk in a microfluidic organ-on-chip setting.

Keywords: Biologics and growth factors, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-292

Engineering Characterisation of Vertical-Wheel™ Bioreactors for Human Pluripotent Stem Cell Expansion and Cardiac Differentiation

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INTRODUCTION: The derivation of human induced pluripotent stem cells (hiPSCs) was a significant breakthrough, allowing for the development of novel therapies for existing diseases. Specifically, the production of hiPSC-derived cardiomyocytes can help combat cardiovascular diseases, which constitute the leading cause of death in the world. Large-scale production of these cells may be performed in Vertical-Wheel bioreactors (VWBRs), which allow for gentle cell suspension with low power input. As such, this work aimed to produce hiPSC-derived cardiomyocytes in VWBRs and to characterise these bioreactors through computational fluid dynamics (CFD), aiming at the scale-up of this protocol.

METHODS: hiPSCs were expanded in 100 mL VWBRs as aggregates, using either a repeated batch or a fed-batch feeding regime and testing dextran sulfate (DS) supplementation, with evaluation of the growth kinetics and cell pluripotency throughout culture. Cardiac differentiation was performed through temporal modulation of the WNT signalling pathway with small molecules, with its performance measured through the percentage of cTNT⁺ cardiomyocytes obtained at the end of differentiation. CFD characterisation of the hydrodynamics and the oxygen mass transfer was performed using simflow for mesh generation and the open-source software OpenFOAM for the simulation itself.

RESULTS: hiPSCs were able to form aggregates and expand in the VWBRs with maintenance of pluripotency during at least 7 days. The best overall conditions (repeated batch with DS) resulted in a maximum cell density of $(2.3 \pm 0.2) \times 10^6$ cells·mL⁻¹ in 5 days, resulting in aggregates with an average diameter of 346 ± 11 μm. Cardiac differentiation led to over 70% cTNT⁺ cells after 15 days. CFD simulations allowed for prediction of bioreactor hydrodynamics and oxygen mass transfer coefficients for a range of stirring speeds and working volumes, which were validated with experimental data. These simulations also showed the VWBRs provide a homogeneous environment for cell growth and that headspace aeration at a 100 mL scale was sufficient for hiPSC expansion.

DISCUSSION & CONCLUSIONS: The expansion and differentiation results suggest the VWBR as a promising platform for hiPSC bioprocessing. Furthermore, the data obtained from CFD can be used for the development of a process for large-scale production of hiPSC-derived cells, with the potential to be used for biomedical applications.

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Keywords: Induced pluripotent stem cells, Scaling up & manufacturing

OP-293

Development of a new microencapsulated system for immunoisolation of insulin-producing cells

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INTRODUCTION: Encapsulation of the pancreas islets of Langerhans is one of the promising solutions for the protection of transplanted endocrine cells in the treatment of type 1 diabetes. The microencapsulating system is a biocompatible matrix that is permeable to nutrients and insulin and impermeable to immune system components, which allows transplant rejection to be overcome without the need for immunosuppressive drugs. However, an ideal capsule that meets the requirements of cytotoxicity, biocompatibility, and permeability has not yet been created.

METHODS: In this study a new encapsulating system for the islets of Langerhans based on alginate and polymer (poly-[2-(methacryloyloxy)ethyl]trimethylammonium chloride (PMOTA)) was created and tested. The capsules were synthesized using the microfluidic method, for which a specialized setup was designed (G.A. Razuvaev Institute of Chemistry, Russian Academy of Sciences). For encapsulation we used the islets of experimental animals. The capsule permeability was assessed by incubating encapsulated islets with FITC-labeled lectins of various molecular weights. To analyze the cytotoxicity of the microcapsule, cell viability was determined before and after encapsulation using live/dead kit staining. To assess biocompatibility and biodegradation rate, microcapsules were transplanted into the omentum or peritoneum of experimental animals. After 7 days, 1 and 3 months the number of washed capsules, their integrity, the degree of cell adhesion and fibrosis were evaluated. The capsules remaining in the tissues were examined using histological analysis. The efficiency of the encapsulated islet cells was evaluated in an animal model with induced diabetes by changes in glucose levels.

RESULTS: It was found that the capsules are permeable to low molecular weight lectins and impermeable to high molecular weight lectins, which allows nutrients and insulin to diffuse through the membrane and limits the permeability of antibodies. The viability of islets after encapsulation after 1 and 3 days was more than 70%. The integrity of microcapsules after washing out was more than 80% and most of the washed capsules were not covered by the surrounding tissues. Histological analysis of the remaining capsules in the tissues revealed little immune response and no fibrosis

around the capsules. The transplanted islet cells in capsules are able to lower blood glucose levels in diabetic rats by at least 18% within 20 days after transplantation compared to a group of rats without treatment.

DISCUSSION & CONCLUSIONS: The technique for encapsulation of the islets of Langerhans can be used as a basis for further development of the microcapsule technology for the treatment of type 1 diabetes without immunosuppression.

Keywords: Advanced therapy medicinal products, Biomaterials

OP-294

Development of an *in vitro* platform to detect tumorigenic events in human Haematopoietic Stem Cells (hHSCs)

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INTRODUCTION: Allogeneic haematopoietic stem cell (HSC) transplant is a curative treatment for a number of diseases such as sickle cell anaemia, haemophilia, thalassemia and severe combined immunodeficiency. The treatments require patients to undergo lifelong pharmacological immunosuppression, and appropriate donors need to be identified to prevent adverse immune responses. In recent years, the use of gene-edited human hematopoietic stem cells (GE-hHSCs) has emerged as a solution [1]. However, gene editing can be accompanied by unwanted events that may lead to cancer [2]. For this reason, a system for efficiently detecting the generation of tumorigenic events is greatly needed.

METHODS: A novel millifluidic optically-accessible bioreactor (MOAB S.r.l., Milano, Italy) [3] was functionalized with a silk fibroin scaffold resembling the physical bone marrow microenvironment and subsequently perfused with an appropriate culture medium. Human CD34⁺ HSCs or CD4⁺ lymphocytes were grown inside, alone, or in the presence of tumour cells. The expansion of specific populations was tested up to three months after culture start. In a follow-up, genetically modified CD34⁺ cells will be subsequently tested for the appearance of clonal expansion.

RESULTS: Long-term culture of primary, human, normal CD34⁺ or CD4⁺ cells was achieved at time-points up to three months. In these conditions, co-culture with tumour cells allowed us to detect the expansion of oncogenic events. Analytical assays, i.e. confocal microscopy and Fluorescence-Activated Cell Sorting (FACS) analyses, were optimized to efficiently detect the appearance of tumour cells. We defined a tumorigenic index of the system as the minimal number of tumour cells, loaded in the co-culture system, that could be detected. As little as 100 tumour cells were clearly detected after three months of bioreactor culture. Current experiments are aiming at detecting as little as one tumour cell and/or the emergence of clonogenic CD34⁺ tumour cells.

DISCUSSION & CONCLUSIONS: We validated an animal-free millifluidic platform, able to expand primary human cells and allow for the detection of rare tumorigenic events. The sensitivity of the system is currently being expanded by the use of state-of-the-art technologies, such as single-cell RNAseq. This model may allow the detection of rare tumorigenic events due to genetic editing in a

reliable, cost-effective and animal-free setting.

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Keywords: Gene editing, Cancer

OP-295

Adipose stem cells with enhanced neurotrophic properties and different cell delivery strategies for peripheral nerve regeneration

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INTRODUCTION: Adipose derived stem cells (AdSC) are known to enhance peripheral nerve regeneration when delivered in nerve conduits through distinct mechanisms. This study aimed at evaluating the synergistic effect of AdSC and nerve growth factor (NGF) in vitro and in vivo¹²³ on axonal outgrowth.

METHODS: AdSCs were pre-treated with NGF (AdSCNGF). Dorsal root ganglia (DRG) were cultured in conditioned medium (CM-AdSCNGF) obtained from AdSCNGF. Axonal outgrowth from DRG was visualized by immunostaining and evaluated by measuring the length and area of neurite outgrowth. For in vivo study, four rats per group were operated creating a 15mm gap on left sciatic nerve. AdSCNGF were either integrated into the wall of FC or injected into the conduit's lumen within a soft fibrin glue solution. Nerve ends were attached by a single suture to the conduit. Functional gait analyses were performed weekly. After four weeks, gastrocnemius muscles and sciatic nerves were explanted. The wet-weight of the muscles was measured and nerve tissue was process for immunostaining and microscopic analysis.

RESULTS: The secretome (CM-AdSCNGF) derived from AdSCNGF exhibited significant axonal outgrowth from DRG explants when compared to controls. In vivo, all fibrin conduits, grafted for bridging the long-gap injuries, showed contraction. Muscle weigh loss was comparable across all groups. The distal nerve cross sections in experimental groups indicate incomplete nerve regeneration, whereas proximal cross sections show an axonal count close to autografts.

DISCUSSION & CONCLUSIONS: The study revealed the synergistic effect of AdSC's derived secretome in the presence of NGF on axonal outgrowth in vitro. The suitability of fibrin conduits for treating long nerve gap injuries is to be evaluated. Although, the 15 mm long gap could not be crossed by regenerating axons within the 4 weeks, the axonal count in proximal part of the nerve and the reduced muscle weight loss indicates the potential of bioactive fibrin conduits for supporting axonal outgrowth over long-gaps.

ACKNOWLEDGEMENTS: We greatly thank the enabling funding support by EUROSTAR (E!10668) to Madduri.

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Keywords: Cell therapy, Microenvironment and niche engineering

OP-296

Cellularized scaffolds of PLA-PEG, alginate, and human chondrocytes for a personalized auricle reconstruction

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INTRODUCTION: External congenital malformations of the pinna, as anotia (completely absence of ear) or microtia (ear not fully developed), have limited options for auricular reconstruction. Nowadays, they have been preferentially treated through auricular reconstruction with autologous tissues. The standard procedure requires costal cartilage removal and carving from several ribs, associated to numerous complications consequence of extended surgeries, and unwanted aesthetic defects in the donor site. To improve the treatments for these patients, tissue engineering replicates the complex anatomy of the ear with a combination of biocompatible scaffolds and autologous or even allogenic human chondrocytes. The aim of this work was to determine a feasible bio-degradable polymer scaffold seeded with human chondrocytes to fully reconstruct an auricle in a safe and reproducible manner.

METHODS: To select the better and feasible strategy for clinical practice, two relevant sources of suitable chondrocytes, from nasal septum and ears, were isolated (CEIC protocol: 2019.100), expanded, and tested in vitro and in vivo in combination with a GMP grade PLA-PEG copolymer (Evonik). To analyze the biocompatibility and potential chondrogenic induction of the material in vivo, the cellularised scaffold with nasal chondrocytes in a non-ear-shaped material was ectopically implanted subcutaneously in Rag2 immunodeficient mice, and analyzed at 3-, 6- and 12-weeks post-surgery. The acellularized material was used as the control group.

A second in vivo model was developed to optimize cellularised inserts made by adding GMP grade alginate to the PLA-PEG and evaluated at 21 days post-surgery by mechanical testing and molecular and histological analysis. Simultaneously, a protocol was designed to fabricate an ear-shaped cellularised material suitable to implant in vivo.

RESULTS: The two sources of chondrocytes tested in vitro were able to maintain a good viability and infiltration in the material (n=2). However, only those chondrocytes from nasal septum showed better distribution in the material and generating distinctive areas with deposition of extracellular matrix (ECM). In vivo time course showed that nasal chondrocytes deposited isolated chondrogenic ECM at 3 weeks. Further time points demonstrated that fibrous invasion and encapsulation of the materials hindering a proper evaluation. Second ectopic in vivo model showed a more elastic behavior in those cellularised alginate inserts compared to the control without cells. Macroscopically, the control without cells showed a calcified appearance, pending of confirmation by molecular and histology analysis.

DISCUSSION & CONCLUSIONS: We were able to characterise a scaffold GMP-quality for a possible

clinical use in auricle reconstruction.

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REFERENCES: Plast Reconstr Surg.1992 Sep;90(3):355-74

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Keywords: Cell therapy, Personalised medicine

OP-297

Investigating the regenerative potential of porcine mesenchymal cells versus myoblasts in a large animal model of stress urinary incontinence

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INTRODUCTION: Stress urinary incontinence (SUI) is a debilitating condition affecting large parts of modern societies. Current therapies are not always satisfactory. We therefore investigated the potential of adipose tissue-derived mesenchymal stromal cells (ADSCs) versus myoblasts (MPCs) in a large animal model of SUI.

METHODS: Cells were isolated from male littermates, expanded, and characterized for expression of lineage marker genes by flow cytometry, immunocytochemistry, and qRT-PCR. Gilts were treated incontinent by electrocautery and balloon dilatation. Characterized cells were co-injected with fluorescent microparticles via needle using a transurethral route in sphincters of incontinent pigs. Functional regeneration was quantified by urodynamics during follow-up. After sacrifice tissues targeted were harvested and investigated by immunohistochemistry.

RESULTS: Porcine MPCs expressed markers for activated satellite cells and proliferating myoblast progenitors such as Pax7, MyoD1, Myf-5, MyoG, Acta-1, Des, and CD56. Upon induction of terminal differentiation, elongated multi-nucleated myofibers were observed. ADSCs expressed mesenchymal antigens including CD29, CD44, CD90, but not MHC class I, CD14, or CD45, and differentiated towards osteoblasts and adipocytes in vitro upon induction. Characterized MPCs or ADSCs were injected in incontinent pigs. Control animals without cell injections maintained a significant sphincter insufficiency for more than 5 weeks of follow up (mean 67.03%; n=6, p<0.05) when compared to animals prior to SUI surgery. Injection of MPCs yielded a trend of accelerated sphincter recovery and reached 81.54% of sphincter function (n=5, not significant). In contrast, injection of ADSCs yielded a significant mean recovery of sphincter function after 5 weeks of follow-up (100.4%; n=6, p<0.05).

DISCUSSION & CONCLUSIONS: Cell therapy of SUI seems more promising upon injection of ADSCs when compared to MPC injections. Isolation of ADSCs from fat seems less invasive when compared to harvesting MPCs from skeletal muscle biopsies. Preparation of ADSCs follows simpler procedures under experimental as well as under clinical (GMP-) conditions. Therefore, therapy with mesenchymal stromal cells such as ADSCs may inherit several advantages in clinical situations.

ACKNOWLEDGEMENTS: This project was in part supported by grant from the DFG and by institutional funding.

Keywords: Cell therapy, In vivo and animal models

OP-298

Open Digital Platform for Managing the Preclinical Translation Process of ATMPs and Biomaterials

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INTRODUCTION: Advanced Therapy Medicinal Product (ATMPs) offer ground-breaking new opportunities for the treatment of diseases that have high global impact. For instance, an ATMP for treating Lower Back Pain is being developed within the scope of Horizon2020 project iPSpine.

Currently, ATMP translation faces long development cycles, high development costs and low success rates. To accelerate and enhance the cost-effectiveness and success of ATMP translation, we propose to support and streamline the preclinical translation phase of ATMPs using Business Process Management technology. For this purpose, we have developed an open digital platform that supports and streamlines ATMP and biomaterial development processes in the context of iPSpine.

METHODS: The open digital platform has been developed using a model-driven approach. The designed models specify the way of working that the platform should support, both considering the tasks to be performed and the data that is collected. The models have been elicited by interviewing iPSpine partners from different fields of expertise (science, regulatory) and by analysing the iPSpine deliverables. The platform has been prototyped using a model-driven, open source software solution for flexible, automated management of business processes.

RESULTS: An open digital platform has been developed for efficiently managing the innovative preclinical translation process of ATMPs/biomaterials. The platform includes template processes and procedures that are best practices identified by iPSpine partners. These templates are easily adjusted to meet the needs of a specific ATMP/biomaterials translation process. Using the templates, the platform enables data collection by researchers for regulatory compliance purposes, particularly data regarding the rationale and set-up of the experiments, methods, results, and decisions based on the data from the experiments. The platform also provides a link to regulatory compliance information (guidelines, directives) provided by the regulatory experts. Thereby, it supports scientists in managing the regulatory compliance knowledge throughout scientific development.

DISCUSSION & CONCLUSIONS: The developed platform offers a basis for managing the translation process of ATMPs. iPSpine partners evaluated the platform positively. They acknowledged the value of having models and a platform that encourages a systematic way of working, which is especially important for guiding future ATMP developments and novice scientists.

Though the platform has been developed specific to the ATMP and biomaterials developed in the iPSpine project, its architecture and template processes can be reused and translated to include other ATMPs and biomaterials. We plan to target this in future work.

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Keywords: Translation and commercialisation (inc. clinical trials and regulatory approval),

OP-299

Making a long story short: brief priming strategies for marrow stromal cell-mediated endochondral bone formation

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INTRODUCTION: Endochondral ossification (EO) is the critical process in bone formation and regeneration whereby cartilage is remodelled into bone. We and others previously succeeded in tissue engineering bone via EO by implanting chondrogenically-primed human marrow stromal cells (hMSCs) in mice¹. Unfortunately, the lengthy phase of in vitro priming (3-5 weeks) is an obstacle for translating such approach to the clinics. Understanding how the chondrogenic priming impacts the various cellular interactions and stages of bone formation will ultimately lead us to speed-up tissue engineering strategies for endochondral bone repair. To address this need we here investigated the effect of chondrogenic priming duration on the dynamics of hMSC-mediated EO, which still remains elusive.

METHODS: hMSC pellets (2x10⁵ cells/pellet) were cultured in the presence of the chondrogenic inducer Transforming Growth Factor(TGF)-β3 for 1-3-5-7-21 days and implanted subcutaneously in athymic mice. In vivo mineralisation was analysed by microcomputed tomography. The pellets were retrieved at several time-points post-implantation (d3-d7-d14-d28-d56-d84) and subjected to flow cytometry to determine the recruitment of innate immune cell subsets, and histological evaluation to monitor the stages of bone formation (3 constructs/time-point/analysis).

RESULTS: After implantation, chondrogenic pellets primed for 7 or 21 days were rapidly encapsulated by fibrous tissue (d3-d7 post-implantation), underwent mineralisation (d7-14) and microvascular infiltration (d28), forming bone with marrow (d56-d84) (N=2 hMSC donors). We observed consistent recruitment of CD11b+Ly6G+CD115- neutrophils and CD11b+F4/80+ macrophage subsets (pro-inflammatory (CD86+), anti-inflammatory (CD163+), tissue repair (CD206+)) for all pellets from d3 onwards. Overall, no significant differences between the two priming conditions were found. Microcomputed tomography demonstrated increased bone volume for 21d-primed pellets (5-fold), while the mineral density was not affected. Next, to determine the minimal priming duration that leads to bone formation, we implanted hMSC pellets chondrogenically primed for 1-3-5-7 days (N=4 hMSC donors). Interestingly, we observed limited bone formation even in the case of TGF-β3 priming shorter than 7 days, but with variable and donor-dependent outcomes.

DISCUSSION & CONCLUSIONS: Our study demonstrates that shortening the chondrogenic priming does not affect the dynamics of endochondral bone formation but reduces the bone volume. We provide proof-of-concept that very brief primings (≤7 days) can lead to bone formation, but further cues will be needed to reduce variability and scale-up bone volume. Our data raise exciting directions for developing translational EO-based tissue engineering approaches for bone defect repair.

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REFERENCES: ¹Farrell E et al. BMC Musculoskeletal Disorders 12,31,2011.

Keywords: Bone and bone disorders (osteoporosis etc), Multipotent (mesenchymal) stem cells

OP-300

Alginate Dialdehyde-Gelatin/Polydopamine Scaffolds Coated With β -Lactoglobulin Permit Subchondral Bone Regeneration

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INTRODUCTION: Subchondral bone damage occurs during late-stage osteoarthritis [1]. To regenerate defects, 3D-printing of polymeric substrates (alginate dialdehyde-gelatin (ADA-GEL)) allows for the tailoring of tissue models and implant designs based on individualized tissue needs. It is also necessary to modify the surface with polydopamine (PDA) in order to improve osteogenic performance [2]. Protein coating treatment using β -lactoglobulin (β -LG) also improves the function and mechanical fixation of scaffolds, as well as promotes osteogenic differentiation [3].

METHODS: We previously described how PDA-coated ADA-GEL scaffolds were fabricated [2]. Protein coating was formed on the surface of the ADA-GEL/PDA scaffolds after they were immersed in ascending concentrations of β -LG (1 mg/ml and 2 mg/ml) for 10 minutes. Physicochemical properties of scaffolds were characterized. β -LG interaction with the outer layer of the scaffold (PDA) was investigated. Sheep bone marrow mesenchymal stem cells (sBMSCs) were used to examine cell-scaffold interactions and osteogenic performance.

RESULTS: The microstructural observation indicates that scaffolds are covered by the layer of β -LG, and the increase in its concentration leads to an increase in protein-protein lateral interactions within the layer, which, in turn, reduces the size and uniformity of the pores. Scaffolds demonstrate a significant increase in absorption capacity as a function of β -LG concentration; however, scaffolds containing 1mg/ml protein present higher stability, as well as a larger density of the biomimetic hydroxyapatite on the surface. Cultures of sBMSCs exhibit a significantly higher rate of cell viability and proliferation, as well as osteogenic differentiation, at a concentration of 1 mg/ml, which arise from the desired availability of protein functional groups.

DISCUSSION & CONCLUSIONS: ADA-GEL/PDA scaffolds coated with β -LG were evaluated to understand how surface modification affects property and osteogenic performance. The study confirms appropriate absorption capacity, controlled degradation rate, and favorable in-vitro hydroxyapatite formation at an optimal protein concentration of 1 mg/ml, suggesting the availability of the functional groups on scaffold surfaces after coating layer formation is at an optimal level. The protein binding study and cell interactions also exhibit that sBMSCs adhered more firmly, proliferated more, and differentiated well when the protein concentration is set at 1 mg/ml, which arise from the desired lateral interaction of proteins at the optimized concentration.

ACKNOWLEDGEMENTS: This work was supported by Alexander von Humboldt Foundation and Bayerische Forschungs Allianz.

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Keywords: Biomaterials, Additive manufacturing

OP-301

BMP2-Incorporated Peptide-Based Bioinks For Promoting Osteogenic Differentiation

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INTRODUCTION: With a growing demand for effective regenerative medicine therapies, more sophisticated bone tissue-engineered in vitro models are required for a better understanding of the fundamental biological processes that underlie regeneration. The state-of-the-art technology of 3D bioprinting aims to achieve well-defined biological structures by the deposition of cell-encapsulated hydrogels or bioinks, in a layer-by-layer manner. A main challenge of 3D bioprinting is the lack of "soft" bioinks with a wide printability window, which offer adequate biofabrication properties as well as a cell-friendly extracellular matrix (ECM)-like microenvironment (1).

METHODS: Self-assembling peptide hydrogels (SAPHs) are fully defined, semi-synthetic hydrogels, which are biocompatible and with tuneable mechanical properties. Therefore, SAPHs are believed to stand as a powerful option with unique properties that make them perfect candidates for this purpose (2). Herein, this research aims to design and explore SAPHs as novel bioinks with incorporated BMP2 for promoting osteogenic differentiation of human mesenchymal stem cells (hMSCs).

RESULTS: To characterise subject hydrogels, rheological analyses, printability and cytocompatibility tests were carried out using oscillatory rheology, extrusion-based bioprinting and hMSCs, respectively. Rheological findings related to good printability in shape fidelity and integrity analyses. We investigated fibroblast and hMSC viability to assess the biocompatibility of the hydrogels. These studies resulted in SAPHs being promising printable and biocompatible biomaterials for extrusion-based 3D bioprinting with good biofabrication attributes.

DISCUSSION & CONCLUSIONS: Up to date, we have successfully developed peptide-based bioinks and assessed the mechanical properties, printability and cell viability of fibroblast and hMSC-encapsulated 3D bioprinted constructs. By means of BMP2-incorporated crystals (PODS®) which provide a sustained release of growth factors, we are currently investigating how bone differentiation can be induced. Our aim is to determine how capable these constructs are to differentiate into physiologic bone phenotype compared to using standard differentiation media. Translated to real-world use, the biofabrication of bone models through 3D bioprinting could result as a powerful tool for in vitro disease modelling and to treat bone conditions such as osteoarthritis in early stages of the disease (3).

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Keywords: 3D printing and bioprinting, Biomaterials

OP-302

Comparative analysis of umbilical cord mesenchymal stromal cells versus their extracellular vesicles for the treatment of inflammatory arthritis

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INTRODUCTION: Due to their immunomodulatory properties, umbilical cord mesenchymal stromal cells (UC-MSCs) have great potential to treat autoimmune disease, such as rheumatoid arthritis (RA). However, to date few MSC clinical products have been approved and problems have arisen with MSC heterogeneity, practicalities of transport and storage, and their response to an inflammatory microenvironment. One potential solution is the use of their extracellular vesicles (EVs) which are small, membrane bound particles with protein and RNA cargo that mimic their parental cell abilities. Nonetheless, many have questioned whether EVs can stand up to the efficacy of their cellular counterparts. Using an antigen induced model of arthritis, we sought to determine the effectiveness of UC-MSCs and their derived EVs in combatting RA-like pathology.

METHODS: Animals were injected with 5×10^5 UC-MSCs, equivalent EVs from the same cell number, or a media control 24 hours post-arthritis induction (n=9 per group). EVs derived from serum free culture were enriched using a sucrose cushion and characterised by Tunable Resistive Pulse Sensing (IZON) for particle concentration and size, flow cytometry (Miltenyi Exosome Detection Kit) and cryo-electron microscopy. Joint swelling was assessed over 72 hours, and histological analysis used to determine arthritis index.

RESULTS: Cells displayed the expected phenotype markers for MSCs and maintained >97% viability in serum free media. EVs from 5×10^5 cells yielded 2.15×10^9 particles/ml (mode size: 126 ± 8.4 nm, mean size: 166 ± 70 nm), with most particles displaying an EV-like morphology of a lipid membrane bilayer and expression of EV and MSC associated markers (CD9, CD63, CD81, CD29, CD44, CD105). Joint swelling decreased for both treatments, in comparison to a media control, but only MSC-EV treated mice achieved significance ($P=0.0002$) at the 48-hour timepoint, with both becoming significant at the 72-hour timepoint. UC-MSCs and EVs achieved a consistent mean lower score in histological analysis indicating reduced immune cell infiltration, hyperplasia, exudate production and cartilage degradation. However, only arthritis index was significant between control and EV treated mice at 72 hours ($P=0.0171$).

DISCUSSION & CONCLUSIONS: Whilst UC-MSCs seemed to improve histopathological signs of inflammatory arthritis, EVs seemed to surpass them. This is backed by the significant reduction in arthritis index suggesting that EVs can not only mimic their cellular counterparts, but may be a superior treatment for RA.

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Keywords: Multipotent (mesenchymal) stem cells, In vivo and animal models

OP-303

Novel strategy to improve mucus penetration of GAG-binding enhanced transduction (GET) gene delivery nanoparticles for inhaled gene therapy of cystic fibrosis

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INTRODUCTION: Cystic fibrosis (CF) manifests mucus accumulation and stasis caused by CFTR mutations. Our previous work developing the glycosaminoglycan (GAG)-binding enhanced transduction (GET) peptides revealed enhanced intracellular delivery of therapeutic cargoes. We then demonstrated that surface shielding of highly cationic GET/DNA nanoparticles (NPs) with polyethylene glycol (PEG) facilitated particle diffusion through human airway mucus, a key delivery barrier to inhaled gene transfer. We here hypothesized that anionic amino acids (AAs) or their polymers would neutralize the cationic DNA NPs to enhance mucus penetration of NPs, providing an alternative to PEG.

METHODS: DNA NPs were prepared via dropwise addition of Gaussia-luciferase (GLuc) reporter plasmid to 1 mM GET at charge ratio 5, yielding cationic NPs. The surface-shielded NPs were prepared via addition of different concentrations or molecular weights (MWs) of anionic AAs or peptides, respectively, after DNA-GET complexation. Hydrodynamic diameter, morphology, and ζ -potential of NPs were determined using a Zetasizer or transmission electron microscopy (TEM). Multiple particle tracking (MPT) was used to evaluate NP diffusion in in CF sputum. In-vitro transfection was assessed using air-liquid cultures of mucus-producing human bronchial epithelial cells or CF-derived patient cell lines.

RESULTS: The NPs shielded by AAs, including glutamic, aspartic, or tranexamic acids, exhibited slightly cationic to anionic surfaces. Different MWs of polyglutamic acid peptides neutralized the otherwise cationic NPs whilst maintaining the hydrodynamic diameters smaller than the average human airway mucus pore size (< 200 nm). TEM confirmed the nanometric and spherical morphology of the surface-shielded NPs. Comparable and normal metabolic activities were observed for NPs regardless of the modification, suggesting the safety of NPs and the coating. MPT analysis revealed markedly enhanced NP diffusion in mucus by the modification. In-vitro transfection efficiency was only marginally reduced, if any, by the surface modification compared to the unmodified NPs, underscoring that masking the cationic surfaces of GET/DNA NPs did not significantly compromise their transfection potential.

DISCUSSION & CONCLUSIONS: We demonstrate here that, unlike PEG, surface modification of GET/DNA NPs with anionic peptides retain their transfection potential whilst rendering the NPs mucus-permeable. Thus, our modification strategy is expected to enhance the transfection efficiency of GET/DNA in the lung following inhalation.

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Keywords: Nanomaterials (inc graphene), Other

OP-304

A novel injectable hydrogel-based drug delivery system for the repair of cartilage defects

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INTRODUCTION: Despite two decades of effort, strategies to regenerate and engineer cartilage tissue have been hampered by the need for a biologically active implanted scaffold to structurally integrate with the native cartilage [1]. The purpose of this study is to develop a versatile injectable and bio-adhesive hydrogel for direct use in fluid-irrigated arthroscopic cartilage repair.

METHODS: An injectable hydrogel system composed of gelatin-methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA) with a base-layer of aldehyde functionalised HAMA (Ald-HAMA) providing an anchor to the defect site via dynamic covalent Schiff-base linkage was synthesised. Injectability of hydrogel solutions were assessed via mechanical and rheological testing. Live/dead staining of constructs with an MG63 osteosarcoma cell-line was used to measure viability. A lap-shear test was utilised to compare the adhesive functionality of the hydrogel to a clinically used fibrin-based tissue sealant. Platelet lysate (PL) was chemically modified to enable covalent bonding to the polymer chains, providing a sustained release of biologically active molecules over time. ELISA and MTT assays quantified the release of growth factors and influence of PL on cell proliferation. Injection of the hydrogel into an ex-vivo cartilage defect model was carried out followed by histological examination of the neotissue.

RESULTS: Rheological and mechanical testing confirmed injectability and gelation after 30s of UV exposure. Mechanical stiffness of hydrogel formulations ranged from 9.96-59.78 kPa. A significant increase in adhesive strength of Ald-HAMA compared to GelMA/HAMA as well as the clinically used sealant was found. Viability of cell laden constructs were ~90% where the proliferation of cells also increased with the addition of PL. The ELISA assay showed a sustained release of growth factors when PL was covalently bonded to the hydrogel. The ex-vivo defect model showed increased cartilage repair for all hydrogels but to a greater extent when PL was incorporated.

DISCUSSION & CONCLUSIONS: Hydrogels exhibited tuneable mechanical stiffness depending on the concentration of polymer and molecular weight of HA. Ald-HAMA displayed greater adhesive strength to tissue than the clinically used tissue sealant, exemplifying the hydrogel's efficacy. PL provided a sustained release of growth factors allowing for the increase in cell proliferation in addition to type II collagen and GAG content found in the neotissue of the ex-vivo defect model. Overall, the developed hydrogel is a promising candidate for the repair of chondral lesions behaving as an effective bio-adhesive scaffold for regeneration of cartilage.

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REFERENCES: Cells. 2019 Oct 23;8(11):1305

Keywords: Hydrogels and injectable systems, Biomaterials

OP-305

A multi-material multicellular 3D *In Vitro* Model for Bone-Tendon-Muscle Interface Regeneration

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INTRODUCTION: Tissue interfaces are transition zones between different tissues. They are essential for transferring load from soft to hard tissues. Although the biology and physiology of interfaces are well known, their development is not entirely understood. Because interfaces have a diminished capacity for regeneration, after surgery, injuries can reoccur, potential graft/prosthetics implanted might not integrate with the joint and a replacement might be necessary [1]. This study aimed to develop a triphasic 3D *in vitro* model to study the bone-tendon-muscle inter-face regeneration.

METHODS: The interface model was made of type I collagen/0.75% (w/v) agarose composite hydrogels with a stiffness increasing from muscle to bone through the addition of hydroxyapatite. The triphasic gels were further crosslinked to improve the stiffness of the bone and tendon sections. Pores for bone, and tendon/muscle fibres were designed to introduce biological relevant surface topography. A growth chamber was developed which separated the cell culture media while allowing cellular crosstalk between the tissue sections. To determine how surface topography affects the interfaces' formation, cells were co-cultured on the 3D model with and without topography. To simultaneously assess cell responses and evaluate the growth chamber's potential to co-culture different cells, the 3D model was cultured in a conventional cell culture plate and compared with the newly fabricated growth chamber.

RESULTS: MG-63, human dermal fibroblast and Sket.4U were used as cell models for bone, tendon, and muscle, respectively. Results show that surface topography influenced the cells' DNA content and metabolic activity. Furthermore, when cultured in the growth chamber, the cell metabolic activity was higher, and the cytotoxicity was lower compared to samples cultured in the standard tissue culture well plate. Hence, the growth chamber was found appropriate for co-culturing different cell types seeded on the 3D interface model. From day 14, cells cultured on the 3D model reached homeostasis and produced new extracellular matrix.

DISCUSSION & CONCLUSIONS: The 3D model and the growth chamber allowed the co-culturing of cells over 21 days. However, the cell seeding densities need to be optimised to promote interface formation.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Biomaterials

OP-306

Comparative bio-assembly of bone cell spheroids in suspension media and pillar array scaffolds

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INTRODUCTION: Bio-assembly of spheroids offers benefits especially in scalable bio-fabrication of artificial tissues. The aim of this study is to comparatively evaluate the bio-assembly of bone cell spheroids in suspension media and pillar array scaffold to produce 3D bone mini-tissues.

METHODS: 50,000 differentiated rat osteoblasts(dRobs) were seeded in 96 well U bottom cell repellent plate to form spheroids in control and mineralisation media. On day 7&14, spheroids were stained with alizarin red for assessing calcium deposits. Suspension media was prepared by cooling a 0.3% gellan gum solution in DMEM through its gel point under shearing, followed by addition of 10% FBS and 1% antibiotic-antimycotic solution. Scaffolds consisting of pillar arrays were designed and printed at different scales with 3DM-XPRO-Grey® resin using an atum3D DLP station5. On day 15, the spheroids were deposited in three columns and two rows into suspension media and scaffolds in both control and mineralisation media conditions. Spheroid fusion was observed on different days and subjected to wax sectioning (10µm thickness) and haematoxylin and eosin(H&E) staining upon fusion.

RESULTS: On day 14, dRob spheroids were observed to produce calcium deposits in mineralisation media as expected.

All the spheroids fused well on day 33 under mineralisation suspension media whereas no adequate fusion observed in control. H&E staining demonstrated that the edges of the spheroids were fused well. However, necrotic core regions were observed throughout the sections.

Spheroids of ~1mm were successfully deposited and held in the smallest scaffolds (0.5 mm distance between arrays) while spheroids in larger sizes were displaced upon media addition. Although it was successful, manual pipetting was highly challenging.

DISCUSSION & CONCLUSIONS: As suspension media affects spheroid position during plate movements and media changes, adequate conditions for spheroid fusion may not be achieved, as our observations showed that spheroids took long time to fuse. Longer culture time with inadequate media changes has resulted in severe necrotic core and would affect normal cell functionality.

However, in scaffolds, spheroid position is fixed and is not disturbed upon media changes. It is expected that the spheroids will undergo fusion in less time than suspension media allowing mitigation of necrotic core. The scaffold method is being customised to setup a semi-automated system which involves a manual micro-manipulator, micro-injector and stereomicroscope for precise deposition of spheroids. Experiments on spheroid fusion using this method are currently ongoing.

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Keywords: Bone and bone disorders (osteoporosis etc), 3D printing and bioprinting

OP-307

AI-supported morphological classification of cell dedifferentiation in a bovine chondrocyte monolayer culture

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INTRODUCTION: The dedifferentiation of chondrocytes is a primary disease mechanism in osteoarthritis. In addition to gene expression changes of cartilage matrix components, morphological alterations are the most prominent markers of chondrocyte dedifferentiation in vivo and in vitro. Previous studies of chondrocyte monolayer culture have quantified these changes in shape using parameters that measure cell area and roundness. This study developed an AI-supported virtual images algorithm, based on a support vector machine (SVM) algorithm, to identify differentiated from dedifferentiated chondrocytes from multiparameter single cell shape measurements.

METHODS: Single cell morphologies of healthy bovine chondrocytes from passages 1–8 were quantified from fluorescent microscopy images by measuring a panel of shape descriptors (area, length, width, circularity, aspect ratio, roundness, and solidity) with a high-throughput automated image segmentation algorithm. Multivariate statistical analyses and virtual image-based binary classification were performed to classify healthy vs. dedifferentiated chondrocytes

RESULTS: The morphology results demonstrated that single chondrocyte shape across passages changed significantly ($P < 0.001$). The analysis of variance between passages suggested that most of the morphological changes happen in cell area, roundness, and solidity. It was hypothesized that a set of chondrocytes from the same passage could be classified accurately as healthy or dedifferentiated, which led to the development of the virtual-images/SVM paradigm, which adds n individual cells together as one datapoint, while retaining all individual variable values. This was repeated multiple time to create a new dataset with the number of data points equal to the number of passages (p) times the number of sampling processes (s). The new data set of dimensions $(n \times v) \times (p \times s)$ can then be assigned two class variables as mappings of the original passage variable and be used for training the SVM model. The classification accuracy was 92% for a classifier. The virtual images paradigm also reveals how many cells are needed for accurate classification of the whole cell population. Here, the algorithm needed at least 15 cells per virtual image to precisely classify healthy vs. dedifferentiated chondrocytes.

DISCUSSION & CONCLUSIONS: The results indicated that single cell morphology descriptors are valuable features to describe and discriminate between chondrocyte phenotypes and that the proposed virtual-images/SVM paradigm based on cell morphological features, is a useful algorithm tool for big-data classifications of single cell phenotypes.

ACKNOWLEDGEMENTS: Nils Lang and Mischa Selig: shared co-first authorship

Keywords: Omics / bioinformatics and systems biology, Cartilage / joint and arthritic conditions

OP-308

Image-based Cellular State Monitoring for Biopharmaceutical Production Cells

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INTRODUCTION: The bio-pharmaceuticals, such as antibodies and protein-based products, are the next-generation medicinal products which ultimately apply the cell culture technologies. Although they now promise for higher therapeutic effect and lower side effects, their manufacturing technology is much complicated than the conventional chemistry-based products. Since the products' efficacy, safety, and quality always greatly rely on their production cells, it is essential to control their quality in every process level in their manufacturing. "How to maintain cell's quality for their product lifecycle" and "how to select suitable cells for further manufacturing consistency" is the recent requirements in cell culture engineers. From such aspect of stabilizing technology for cell culture and production, image-based analysis can offer real-time information to feed back for the culture control and optimization. Our group has been investigating the use of microscopic images to non-invasively assess, record, and monitor cell's quality during the cell culture process in manufacturing [1, 2]. In this work, we have expanded our method to evaluate the Chinese hamster ovary (CHO) cells and their delicate differences.

METHODS: CHO cell lines with or without the production, and their metabolism regulator compounds were used to set different quality conditions. For their morphological analysis, BioStation CT (Nikon) were used for punctual and continuous imaging. Autofocusing condition was optimized, and all the time-course image data were analyzed by our own image processing algorithms using Python. Extracting multiple morphological parameters of time-course, the quality differences were analyzed and visualized by clustering and machine learning classifiers.

RESULTS: Our image analysis of morphological parameter extraction succeeded in extracting explainable morphological features not only from the adhesive CHOs but also suspended CHOs, to discriminate their production performances and metabolisms.

DISCUSSION & CONCLUSIONS: By our data, we found that even with the suspended cells, our morphological analysis succeeded in analyzing the delicate differences in CHOs, therefore can be applied to other suspended type cells in cell therapeutic products advancing in the recent regenerative therapies.

ACKNOWLEDGEMENTS: Authors are grateful for financial support from Medical Research and Development Programs from the Japan Agency for Medical Research and Development, AMED.

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Keywords: Biologics and growth factors, Enabling technologies

OP-309

High-throughput rational design of artificial tissue moulds using a biophysical in-silico model and machine learning

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INTRODUCTION: We use a microscopic biophysical model for self-organization and reshaping of lab grown polarised tissues to identify candidate moulds for growth of artificial tissue from hydrogels. Designing moulds and scaffolds that lead to specific patterns of self-organisation in artificial tissues is a slow and painstaking effort. Computational models could help with this process. We use a biophysical model of self-organisation in polarised tissues that includes both extracellular matrix (ECM) and cells. The model uses a mix of contractile networks and dipole orientations (CONDOR) to simulate the feedback between microscopic active forces mediated between cells and ECM, and macroscopic forces that develop on tissue length-scales. When large numbers of cells act together these forces drive, and are affected by, macroscopic-scale self-organization and reshaping of tissues in a feedback loop.

METHODS: We use the CONDOR model [1], which consists of a contractile network representing the ECM, that interacts with large numbers of cells via dipole forces. By simulating the interactions between large numbers of cells and the ECM in this way, we track both individual cells and the ECM, like the situation within tissues. This allows us to describe the macroscopic self-organization and reshaping of tissue. We solve the model using simulated annealing, Monte Carlo and machine learning techniques. This allows high-throughput simulation of large numbers of tissue moulds.

RESULTS: Close agreement has previously been shown with experiments on artificial neural tissue [1]. We used the CONDOR model to make high-throughput calculations for a wide range of computer-generated moulds. We identified a fitness function so that we can identify (computationally) the relative merit of moulds for growing artificial neural tissue. Using this process, we have identified candidate mould designs for growth of artificial neural tissue with favourable properties.

DISCUSSION & CONCLUSIONS: Feedback between cells and ECM is a key factor in driving macroscopic self-organization and reshaping of tissue. The CONDOR model has previously been shown to match well with experimental data. We demonstrated proof-of-concept for using the model for high-throughput rational design of artificial tissues. Model extensions will lead to further tools for rational design of moulds, scaffolds, and other approaches for growing artificial tissue.

ACKNOWLEDGEMENTS: We acknowledge support from the STFC Impact Acceleration Account

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Keywords: In silico models, Hydrogels and injectable systems

OP-310

Automated Biofabrication of Multicellular Human Tendon Microphysiological Systems Within a Biomimetic Fibrillar Support Platform

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INTRODUCTION: Tendinopathies are not well understood diseases for which current treatments have poor recovery outcomes. Therefore, relevant *in vitro* models to study these diseases and develop better treatments are highly needed. Recently, we proposed an innovative strategy that allows the automated 3D writing of microphysiological systems (MPS) embedded into its own biomimetic fibrillar support platform based on the self-assembling of cellulose nanocrystals (CNCs)¹, which we believe is promising for tendon modeling. Bioinks based on decellularized extracellular matrix (dECM) are able to closely recapitulate the rich microenvironment of the tendon, whose extracellular matrix (ECM) is responsible for activate and regulate several signaling pathways controlling cell behavior. Here, we explored our CNC platform as support for writing humanized *in vitro* tendon models using tendon dECM-based bioinks to closely recapitulate the biophysical and biochemical cues of tendon cell niche and self-induce the tenogenic differentiation of stem cells.

METHODS: The dECM bioink hydrogel was prepared from decellularized porcine flexor tendons, and the easily accessible hASCs were used as cell source. The bioink was directly printed within the CNC fluid gels used as support media for freeform bioprinting of embedded constructs and their *in vitro* maturation. To evaluate the effects of cellular crosstalk with endothelial cells, tendon constructs were co-printed with compartmentalized microvascular structures. The CNC embedded 3D structures were characterized by histochemistry, qRT-PCR and immunofluorescence analysis.

RESULTS: The developed MPS showed high cell viability, proliferation, and alignment during culture up to 21 days. Gene and protein analysis showed upregulation of the most important tendon related markers on tendon constructs, which were boosted when co-cultured with endothelial cells. In co-culture system, chemotaxis induced endothelial cells migration toward the tendon compartment, but without showing significant infiltration in these structures.

DISCUSSION & CONCLUSIONS: The synergy between dECM cues and printed patterns induce cells organization similar to tendon tissues. Besides cells organization, gene and protein expression demonstrated that the biophysical and biochemical cues of dECM induce hASCs commitment toward tenogenic phenotype, while the chemotactic effect in the presence of endothelial cells boosted hASCs tenogenesis, emulating tendon development stages.

Overall, the proposed system might be promising for the automated fabrication of organotypic tendon-on-chip models that will be a valuable new tool to study tendon physiology, pathology, or the effect of drugs for the treatment of tendinopathy.

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Keywords: 3D printing and bioprinting, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-311

Human three-dimensional multicellular liver platform for drug screening

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INTRODUCTION: The pharmaceutical industry is currently facing increasing challenges in drug discovery due to long drug development times combined with low clinical trial success rates. Over 20% of approved drugs suffer from market withdrawal for severe liver injury at therapeutic doses, becoming a significant cause of death worldwide, with an estimated 2 million patients in the United States. Three-dimensional (3D) cellular models, obtained by mixing cells with a biomaterial in a pre-specified structure, are emerging as a physiologically relevant cellular microenvironment holding a great promise for drug safety and efficacy in the early phase of drug development.

METHODS: Here, we developed human 3D livers combining hepatocytes (HepaRG), hepatic stellate cells (HSCs) (LX-2), and immune cells (THP-1), the three main cell types of the liver, in a 2:1:1 ratio. We used a well-established mixture of gelatine methacryloyl (GelMA) and carboxymethyl cellulose methacrylate (CMCMA), a biodegradable and non-biodegradable material, respectively. Lithium Phenyl(2,4,6-trimethylbenzoyl)phosphonate was used as a photo-initiator. Cells were combined with the GelMA-CMCMA mixture (1:1) and exposed to UV light for 30 seconds. The 3D livers were kept in culture for up to 30 days in serum-free medium. They were challenged with acetaminophen and LPS, two well-described hepatotoxic compounds, to recreate the pathophysiological phenotype of liver damage in vitro.

RESULTS: The 3D livers did not show any signs of distress while resembling many characteristics of the native healthy liver. We evaluated the cell viability, morphology, and gene expression of hepatocytes, HSCs, and immune cells in the 3D livers during the 30 days. The LPS and acetaminophen challenge induced extensive liver damage characterized by HSC activation and proliferation. These cells acquired the typical myofibroblast phenotype of activated HSC in vivo. Moreover, we assessed gene expression markers of hepatocyte functionality, such as albumin, OTC, and CPS1, as well as the CYP3A4 enzyme activity, which were impaired upon treatments. The inflammation level of the system was measured by the transition from the THP-1 monocytes to macrophages releasing proinflammatory cytokines (such as IL-1 β , IL-6 and TNF- α) into the medium.

DISCUSSION & CONCLUSIONS: Human 3D livers are suitable for long-term cultures and can be used to test the liver toxicity of drugs, as has been demonstrated with LPS and acetaminophen. These results also validated this multicellular system as a valuable tool for measuring liver damage and inflammation, as the 3D livers successfully displayed the characteristics of liver damage in vitro.

Keywords: Biofabrication, In vitro microenvironments

OP-312

3D micro scaffolds as a frontier tool to model the EMT phenotype in cancer cells

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INTRODUCTION: Breast cancer is the most frequently diagnosed cancer, and the leading cause of death, among women. Its onset is associated with phenotype transition from epithelial to mesenchymal (EMT), a process generating cancer stem cells (CSCs)[1, 2]. Markers of EMT are loss of cellular contacts, β -catenin nuclear localization, cytoskeleton reorganisation, vimentin expression and increased motility[3]. There are several factors of the tumour microenvironment known to influence EMT, such as hypoxia, immune response and stiffening of the extracellular matrix[4]. We developed a culture substrate, called Niche, which is a custom-made, microporous, optically accessible 3D scaffold fabricated by 2-photon laser polymerization[5], in which cells adhere and grow in a spatial configuration different from the spheroid standard culture condition. Here, we analyse the ability of this substrate to sustain the EMT phenotype in CSCs.

METHODS: Malignant breast cancer cell lines (MCF7/MDA-MB-232) were grown in different cell culture conditions: on 2D flat glass coverslips, on 2D soft collagen based-hydrogels (with Young's modulus of 10Pa), in the 3D Niche, and in conventional 3D spheroids. We used a pharmacological control to destroy actin cytoskeleton and reduce intracellular tension. Immunofluorescence assays were used to characterize the organization and localization of EMT-key markers: actin, vimentin, and β -catenin. The role of the various substrates in modulating cell migration was investigated by time-lapse microscopy.

RESULTS: Results show that the Niche and the pharmacological control limit the cell capability to migrate. The actin fibers number, related to intracellular tension, is highest on flat, and reduced in the Niche and spheroids. Vimentin is less expressed in the Niche than 2D glass and protein localization in the Niche is similar to spheroid samples. Only the Niche and the spheroids influence nuclear β -catenin translocation.

DISCUSSION & CONCLUSIONS: Our results suggest that the Niche, influencing cellular adhesion properties, can modify key parameters related to cancer phenotype, primarily cell motility. This substrate also guarantees optical access to the samples to monitor in real-time the phenomenon evolution. Therefore, the Niche is a valuable tool for 3D-cancer-models and pharmacological research in vitro. We are now using this substrate to evaluate how mechanobiology can improve knowledge and research approaches related to cancer biology.

ACKNOWLEDGEMENTS: ERC-project BEACONSANDEGG G.A.10105312

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Keywords: Microenvironment and niche engineering, In vitro microenvironments

OP-313

CombiCHEM: screening for effective combinations of anti-cancer drugs

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INTRODUCTION: Chemotherapy is an essential component of malignant tumour treatments, but tumour resistance to single agent therapies occurs frequently. Inter-patient and intra-tumour heterogeneity of the tumour microenvironment (TME), including the presence of different cell types and composition of the extracellular matrix (ECM), impact the potential effectiveness of combinatorial treatments. Therefore, there is a need for combinatorial drug screening assays that can identify synergistic drug combinations and concentrations while including key aspects of the TME. To address this, we present CombiCHEM, a novel combinatorial anti-cancer drug assay, which is based on a recently published antibiotic screening device¹.

METHODS: The CombiCHEM drug screening assay consists of a 3D-printed cell culture insert with three individual drug reservoirs each loaded with a drug of interest dissolved in a hydrogel. The drug-loaded CombiCHEM insert is inverted and submerged into a standard cell culture dish containing cancer cells, encased in the same gel. Diffusion of drugs from the reservoirs establishes dynamic and overlapping drug gradient landscapes, which the cells will be exposed to. Cell viability is monitored by live imaging of fluorescent apoptosis and cell death reporters. Initial assay validation was performed using staurosporine (apoptosis-inducer), and MDA-MB-231 breast cancer cells. Subsequent validation steps used clinically relevant chemotherapeutic combinations confirmed to exhibit additive or synergistic effects.

RESULTS: Gradient landscape formation was visualized by dissolving fluorophores with similar molecular weight to drugs-of-interest in the reservoir hydrogels. Time lapse fluorescence microscopy revealed overlapping dynamic gradients. Subsequently, MDA-MB 231 cells were exposed to staurosporine gradients and cell responses were recorded. Similar to the fluorophore gradients, gradients of apoptosis and cell death signals formed over time. Next, an image analysis strategy was assessed to detect the effect of drug combinations. Finally, clinically relevant drug combinations were used to analyse cell responses to combinatorial treatment effects.

DISCUSSION & CONCLUSIONS: The CombiCHEM assay is a novel anti-cancer combinatorial drug screening assay. An essential feature is the establishment of dynamic drug gradients in hydrogels. In the future, fine-tuning of gel compositions, 3D bioprinting based ECM patterning and co-culture of relevant cell types will enable mimicking of the pathological ECM composition in the TME and will provide a better model for tissue-specific drug responses.

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Keywords: 3D printing and bioprinting, Cancer

OP-314

Bioengineered in vitro 3D model of lung cancer tumor microenvironment with tunable extracellular matrix to investigate the pre-metastatic niche formation in distant organs

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INTRODUCTION: Lung cancer metastasis is the leading cancer-related mortality in both men and women worldwide, there is an urgent need to identify effective therapeutic targets in order to improve the disease outcome and overall survival rate (1). In particular, understanding the interaction between stroma within the primary tumor microenvironment and changes in stroma stiffness, on the influence of the pre-metastatic niche (PMN) formation, along with cancer cell signalling from the primary tumor site to distant organs facilitating secondary growth, remains unclear (3). The use of 3D in vitro models, particularly in cancer research, is advantageous as they can mimic the 3D in vivo characteristics of the tumor microenvironment, highlighting the potential to develop a representative in vitro 3D model to study PMN formation and identify new treatment targets for metastasis. This project aims to develop a 3D gelatin methacryloyl (GelMA) hydrogel model of lung cancer in which to investigate the role of tumor microenvironment stiffness in PMN formation within lung cancer.

METHODS: GelMA hydrogels formulations with varying stiffness for 3D in vitro applications were fabricated using previously optimised protocols (4). Hydrogel characterization and biocompatibility were performed using mechanical testing, microscopy, and AlamarBlue assays for the A549 lung cancer cell line.

RESULTS: Alterations on the photoinitiator Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) concentration increased the stiffness of hydrogels in a concentration-dependent manner to mimic healthy, pre-cancerous, and cancerous lung tissue properties. An increase in hydrogel stiffness affected cell growth and A549 cells were less metabolically active on stiffer hydrogels. Development of hydrogels infused with lung cancer cells have been developed and is under investigation.

DISCUSSION & CONCLUSIONS: In conclusion, this study demonstrates that GelMA hydrogel is a suitable material to support the growth and proliferation of A549 cell lines. Furthermore, the results demonstrate the potential of these hydrogels to act as efficient platforms to investigate the role of tumor microenvironment stiffness in PMN formation in lung cancer.

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Keywords: Cancer, Biomaterials

OP-315

Assembloid-based 3D melanoma models

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INTRODUCTION: 3-Dimensional (3D) in vitro models of tumours are increasingly considered as valid alternatives to animal models for basic research and pharma testing due to economical and animal welfare reasons. But their performance depends ultimately on their biosimilarity to native tumours, ie, on their ability to recapitulate the cellular and matrix interactions within the tumour microenvironment that lead to representative levels of tumour aggressiveness and invasion. In this work, we describe the creation of 3D melanoma models with higher complexity using an assembloid-based methodology.

METHODS: Different multicellular spheroids representing different stromal compartments of melanoma were created and allowed to assemble into higher complexity melanoma-like structures. Histoarchitecture, gene expression and invasiveness were evaluated and the results were compared with those of less complex multicellular spheroids.

RESULTS: The assembloid method allowed the formation of self-organizing melanoma models, with a histoarchitecture close the one of native melanoma. We demonstrate that in these models tumour cells display stem cell-like features such as upregulated pluripotency markers. Assembloid models embedded in a 3D matrix displayed high levels of invasiveness regulated by stromal cells. Finally, the developed models were demonstrated to possess sensitivity to the anticancer drug doxorubicin.

DISCUSSION & CONCLUSIONS: This is, to our knowledge, the first application of the assembloid method for the creation of 3D melanoma models. The observed results suggest that, in comparison with traditional multicellular spheroids, assembloid-based models of melanoma more closely mimic native tumour biology, supporting their use as potential alternatives to animal models for drug testing and basic research.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Cancer

OP-316

Human skin equivalents with perfusable hierarchical vasculature

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INTRODUCTION: Anatomically-correct models of human skin are key to understand skin homeostasis and regeneration. However, currently used in vitro skin cultures are predominantly limited to a collagen-based dermis with fibroblasts and an epidermis with keratinocytes. More sophisticated skin models are needed to properly capture the skin's cellular heterogeneity and complex anatomy in vitro to investigate skin diseases and regeneration. Such a model of the skin is difficult to set up due to its cellular heterogeneity and intricate interaction between the substructures. Therefore, the next generation of in vitro skin models needs to provide an accurate depiction of homeostatic, aging, or injured skin. Since current full-thickness skin equivalents lack functional vasculature, the establishment of functional circulation, perfusion, or inclusion of the immune system is not possible. As a solution, we here engineered a vascularized 3D full-thickness in vitro skin model (vascuSKIN) including an epidermis, and a dermis with vasculature by combining vascular self-assembly and full-thickness skin equivalent culture.

METHODS: Hierarchical macrovascular trees were manufactured from UpFlex material using 2-Photon Polymerization (2-PP) printing (UpNano, Vienna, Austria). The VascuSkin device and periphery were designed using Fusion360 Software, molds were printed via additive manufacturing and devices were fabricated via soft lithography. Human dermal fibroblasts, endothelial cells, and adipose-derived stem/stromal cells were embedded in hydrogel co-gels containing fibrin and collagen I before seeding of keratinocytes and epidermal differentiation at air-liquid interface culture for 14 days. Skin equivalents were fixed in 4% buffered formalin, embedded in paraffin, sectioned, and stained using immunofluorescence for histological analysis.

RESULTS: We first established fibrin-collagen co-gels as an alternative to collagen-only hydrogels permissive of self-assembled vascularization and limited contraction. In addition, histological analysis showed proper epidermal stratification including expression of keratin 14, keratin 10, and loricrin. Importantly, endothelial cells embedded into the dermis formed a continuous, lumenized

microvasculature as visualized by CD31 staining. For perfusion culture, we engineered a porous hierarchical vascular tree using 2-PP printing using a novel material. The material is biocompatible and supports culture of endothelial cells as well as self-assembled microvasculature.

DISCUSSION & CONCLUSIONS: In summary, these results show the successful establishment of full-thickness skin equivalents with vascular structures using collagen-fibrin co-gels and human cells. Furthermore, our model resembles physiological skin by including a dermis and an epidermis that consists of the essential cell types associated with wound healing.

ACKNOWLEDGEMENTS: Austrian Science Fonds grants P 35382 (M.O., B.B.), and P 35268-B (J.G.), and the Lorenz Böhler Gesellschaft grant LBF 6/21 (B.B.).

Keywords: Skin, In vitro microenvironments

OP-317

Development of a perfusable vascularized in vitro skin model from hiPSC derived skin organoids for disease modelling and infection studies

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INTRODUCTION: Mimicking the complex structure of human skin in vitro is a major challenge. The reason is that the largest organ of the human body consists of several layers: epidermis, dermis and hypodermis, which various cell types and structures such as hair follicles are located. Existing simple models lack both complexity and in addition a perfusable vascularization. Complex models could be used to study infectious diseases such as sleeping sickness in humans caused by the African parasite Trypanosome Brucei. These models could additionally be a test platform for modeling diseases such as skin cancer or skin fibrosis.

METHODS: Using human induced pluripotent stem cells (hiPSC), it is achievable to generate organoids that replicate this complexity. Combining skin organoids with a perfusable vascularized hydrogel can more accurately represent the physiology of the skin than previous in vitro models. In parallel, the development of a vascularized perfusable hydrogel using a sacrificial scaffold and a hydrogel composed of GelMA and ColMA in a bioreactor is realized.

RESULTS: In the hiPSC derived skin organoids we identified several skin-specific cell types such as keratinocytes (CK5), dermal cells (vimentin), adipocytes (nile red) and complex hairpeg formation (indicated by the expression of CK5, CK17). Skin organoids were stung at day 140 by flies infected with Trypanosoma brucei. At the injection site, we saw infiltration of Trypanosoma brucei expressing the fluorophore tdTomato into the dermal layer, without infiltration into the epidermal part. At 7 days post infection, the parasites have distributed throughout the complete organoid and accumulate in specific areas of the dermal layer without infiltrating the epidermal parts.

Vascularization will be realised with hiPSC derived vascular organoids. We identified a vascular network (VE-Cadherin), with endothelial cells (CD31), pericytes (NO-GC, PDGFR β) and smooth muscle cells (SMMHC).

DISCUSSION & CONCLUSIONS: This complex skin model will be used to further investigate the mechanism of invasion of the parasite into the human blood stream as well as other pathologies.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Skin

OP-318

Towards the role of cellular senescence on cell mechanics and ECM formation

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INTRODUCTION: Transient cell cycle inhibition in G1 or G2 phase is an important basic means to prevent accumulation of genomic alterations. However, long-term cell cycle arrest, e.g. as a consequence of prolonged cellular damage, leads to cellular senescence. Senescent cells accumulate in aging organisms and a persistent, long-term presence is detrimental as it compromises the functionality of surrounding cells and drives tissue degeneration. Recent work, however, highlights the ambivalent and even beneficial role senescence has during wound healing. Here we investigated the role of damage- and non-damage-induced cellular senescence on tissue formation and contraction using a recently established in vitro wound healing model [1]

METHODS: Primary human dermal fibroblasts were driven into cellular senescence either by DNA damage through application of Mitomycin C (MMC) or by conditional over-expression either of p16 or p21 cell cycle inhibitors. Cells were analyzed by confocal imaging for their adhesion behavior (focal adhesions) and by traction force microscopy for their tensional state. Furthermore, ECM and in particular fibrillar collagen formation was analyzed in 3D using a macro-porous collagen scaffold as a cell carrier material.

RESULTS: Depending on how senescence was induced, this affected focal adhesion formation and cell force generation. Cellular senescence was found to modulate macroscopic tissue formation and contraction in vitro as a result of an altered cell mechanics and a regulated ECM protein expression pattern. DNA-damage-mediated senescence hereby solely led to reduced expression of collagens and lysyl oxidases which ablated any tissue formation potential despite highly increased cell forces.

Persistent cell cycle inhibition in contrast held beneficial effects for tissue formation and contraction. **DISCUSSION & CONCLUSIONS:** This study highlights the mechanical contribution of senescent cells during wound healing as well as the inter-dependency on the ability to form a tensioned and load-bearing ECM network. These observations point to an active and potentially beneficial role of aspects of cellular senescence during tissue formation that competes with and eventually succumbs to catabolic factors as a result of the DNA damage response.

ACKNOWLEDGEMENTS: This work was funded by the DFG (CRC1444 and FOR2165).

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Keywords: Disease models, Musculoskeletal (inc ligament / tendon / muscle / etc)

OP-319

Chromatin modulation in macrophages as a therapeutic strategy to regulate inflammatory genes in diabetic impaired wound healing

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INTRODUCTION: Effective repair of cutaneous injury requires a transient inflammatory response during which macrophages are recruited from the periphery to the wound site. As healing progresses from the inflammatory phase to the tissue remodelling phase, macrophages switch from an M1-like, pro-inflammatory phenotype to an M2-like phenotype that promotes wound resolution. In the diabetic chronic wound, however, this switch is absent or delayed. Instead, myeloid cells continue to express M1-associated genes and do not transition to an M2-like pro-resolution state. Epigenetic changes to macrophages occur during ageing and in pathological conditions such as diabetes. These changes are associated with altered macrophage differentiation and function contributing to chronic inflammation, resulting in poor tissue repair and regeneration and delayed wound healing.

METHODS: We have used adoptive transfer of macrophages, transient gene therapy and protein transduction of the transcription factor Hoxa3 in mice in vivo and in human patient-derived macrophages in vitro to reprogramme cells from the diabetic environment to promote wound healing. In addition, we have used flow cytometry and next generation sequencing to identify intrinsic factors regulating macrophages during wound healing as well as identify factors modulated by Hoxa3 treatment.

RESULTS: We have identified several chromatin modifying enzymes in macrophages that appear to play a role in the transition from the pro-inflammatory to pro-healing phenotype that may contribute to longer term changes in macrophages in pathological environments. Specifically, we have determined that these changes result in priming of macrophages and their progenitors to respond aberrantly to pro-inflammatory signals in the wound environment. Moreover, we have found that chromatin alterations are associated with aberrant differentiation of macrophages and their progenitors. We have also found that Hoxa3 can be used as a therapeutic factor and when provided exogenously can control macrophage differentiation and function through interactions with epigenetic regulators.

DISCUSSION & CONCLUSIONS: Therapeutic manipulation of macrophage epigenetics with biologics such as Hoxa3 can induce long-term changes to these cells and impact tissue repair and regeneration in diabetes and has significant relevance for people who have reversed their condition.

Understanding how these mechanisms affect wound healing is critical to developing future therapies that work not just in diabetes but in ageing tissues as well.

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Keywords: Biologics and growth factors, Wound healing

OP-320

Dermal extracellular matrix extracts for wound healing: a pleiotropic trigger

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INTRODUCTION: Extracellular matrix (ECM) role is defined by direct cell-ECM interactions and biomechanics and also by its capacity to store biochemical cues that are vital in tissue's repair. With this in mind, an in-house method was devised to obtain extracts comprised of structural ECM components (strECM) and enriched in soluble ECM-derived factors (sECM). Herein we hypothesised that each ECM fraction may trigger different biological functions in multiple cell types, objectively conferring them therapeutic and biomimetic potential.

METHODS: To prove the concept, we used human dermal fibroblasts (hDFBs) to obtain the ECM extracts, prepared by fractioning cultured cells' own ECM. Extracts were analysed by mass spectrometry to obtain a proteomic profile and then regarding their in vitro functionality. Human dermal endothelial cells (hDMECs), keratinocytes (hKCs) and dFBs, were used to confirm the features identified by the proteomic profiling. The effect of the extracts over cell adhesion (focal adhesion formation) was analysed. A Matrigel assay was used to evaluate a potential angiogenic effect of the extracts. Moreover, hKCs migration and ability to differentiate and form a stratified epidermis was assessed. Finally, matrix (Collagen, elastin, GAGs) deposition by hDFBs and metalloproteinases (MMP 1, 2, 9) secretion and activity were measured.

RESULTS: Proteomic analysis revealed that strECM and sECM complement each other, preserving the native ECM protein profile. The GO accessions linked to each fraction allowed pinpointing the specific cues provided by either of them. strECM was mainly comprised of components that were able to promote cell adhesion and spatial organization. On the other hand sECM proteomic profile revealed factors that play a role in wound healing such as angiogenesis, ECM remodeling and re-epithelialization. A dose-dependent response was observed regarding the formation of tubular structures in the angiogenic assay. The presence of sECM leads to a significant increase in the migration and proliferative ability (Ki67) of hKCs while maintaining their phenotype. Finally, sECM led to enhanced collagen, elastin and GAGs deposition by hDFBs while increasing the expression of MMPs.

DISCUSSION & CONCLUSIONS: Our results validate the hypothesis that each ECM fraction effectively triggers different biological functions in multiple cell types. Overall the presence of sECM boosted the major cellular mechanisms that lead to successful wound healing, while strECM provides cues for cellular adhesion and organization.

This study supports the use of ECM extracts as a wound healing enhancer, which might aid in the development of future therapies or improve the biomimicry of ECM-based 3D tissue models.

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Keywords: Wound healing, Skin

OP-321

Functionalisation of a Collagen-Based Scaffold for Nucleic Acid Delivery in Wound Healing Applications

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INTRODUCTION: Diabetic foot ulcers (DFUs) are a type of chronic wounds that constitute a disabling complication of diabetes. Hyperglycemia causes impaired vascularization, reducing healing potential, leaving wounds open and increasing risk of infection. Collagen-based scaffolds are biomimetic templates that have shown promise to facilitate tissue repair and remodeling in DFUs¹. However, DFUs present extensive gene dysregulation and such scaffolds require further functionalization to promote healing. To address this challenge we propose that such collagen-based scaffolds may be functionalized to act as reservoirs for nucleic acid delivery to direct pro-regenerative gene expression². Therefore, this work focused on the functionalization of a collagen-glycosaminoglycan (CG) scaffold with non-viral vectors carrying pro-angiogenic nucleic acids to promote healing and closure of DFUs

METHODS: Human dermal fibroblasts (hDFs) were transfected with GAG-binding enhanced transduction (GET)³ peptide and lipofectamine 3000 (positive control) carrying the pro-angiogenic plasmid encoding VEGF (pVEGF) in 2D, before moving to an in vitro 3D (CG) scaffold. Biocompatibility in 2D was assessed through an alamarBlue metabolic assay. Protein upregulation was quantified through ELISA. hDF-seeded gene-activated CG scaffolds were also characterised through alamarBlue and ELISA with hDF morphology on the gene-activated scaffolds was also characterised by fluorescent staining and confocal microscopy.

RESULTS: GET/pVEGF-treated hDFs displayed higher metabolic activity (alamarBlue) and protein expression (ELISA) in 2D than those treated with lipofectamine/pVEGF. GET/pVEGF gene-activated scaffolds (CG-GET) metabolic activity supported the 2D results while VEGF production was comparable to the scaffolds with lipofectamine/pVEGF. Additionally, confocal microscopy displayed reduced number of viable cells for the CG-lipofectamine scaffolds compared to CG-GET and CG scaffolds, confirmed by image analysis and quantification.

DISCUSSION & CONCLUSIONS: Here, we demonstrate the successful gene-activation of CG scaffolds with a peptide-based vector (GET) carrying pVEGF as a promising platform for enhanced vascularisation in wound healing. This gene-activated scaffold resulted in enhanced cell proliferation and pro-angiogenic activity. hDF proliferation on CG-GET scaffolds outperformed that of CG-lipofectamine while providing comparable pro-angiogenic protein expression. Taken together, this data indicates that hDF proliferation and VEGF production can be enhanced through GET/pVEGF

transfection in a CG scaffold, making it suitable for the treatment of chronic wounds. Future work will determine the "*in vivo*" effect of CG-GET in key wound healing processes such as re-epithelialisation and angiogenesis.

ACKNOWLEDGEMENTS: Science Foundation Ireland / Advanced Materials and Bio-Engineering Research. Grant: SFI/12/RC/2278_P2

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Keywords: Biomaterials, Gene therapy

OP-322

How can we administer mesenchymal stem/stromal cell secretomes for skin wound healing?

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INTRODUCTION: Mesenchymal stem/stromal cells (MSC) are a promising tool in the treatment of skin wounds as they secrete regenerative growth factors, cytokines and chemokines that can advance skin wound healing through paracrine activity on endogenous cells. However, the delivery of such MSC-derived wound healing factors remains problematic. This study has examined how culture conditions and duration may influence the paracrine activity of MSCs, using a cell delivery system that has entered the clinic for skin wounds.

METHODS: Mouse MSC (ST2 cells) were seeded into T75 flasks, incubated overnight to adhere, then the medium changed for either serum-supplemented or serum-free medium for 24-72 hours at which point MSC culture-conditioned media (MSC CM) were harvested. MSC CM also were obtained from MSCs cultured on MySkinTM scaffolds for 48 hours. These various MSC CM were used to treat mouse dermal fibroblasts (L929 cells), where dermal fibroblast proliferation was determined using MTS assays and dermal fibroblast migration was examined using scratch assays. Further, MSC CM was used to coat culture plates or the MySkinTM scaffold to see if this influenced dermal fibroblast or MSC adhesion. Cell adhesion, proliferation (and viability on the MySkinTM scaffold) were determined by MTS assays and LIVE/DEAD staining. All experiments were performed on at least 3 separate occasions.

RESULTS: Culturing MSCs in serum vs serum-free conditions had little effect on their viability, but resulted in fewer cells when serum-free. Serum-free MSC CM harvested after 24hr and 72hr significantly increased dermal fibroblast proliferation versus control medium. Dermal fibroblast scratch wound closure was significantly enhanced by 48hr serum-free MSC CM generated in tissue culture flasks or on the MySkinTM scaffolds versus control medium. MSCs cultured on MySkinTM scaffold in serum-free conditions were greater than 70% viable for up to 3 day. Further, pre-coating MySkinTM with 48hr serum-free MSC CM increased MSC adhesion and proliferation versus control medium.

DISCUSSION & CONCLUSIONS: This study has shown both that culturing MSCs under different conditions and duration affects their skin wound healing paracrine activity on dermal fibroblasts, and also that these paracrine effects may be delivered through MSCs cultured on a cell delivery system that already is in clinical application for skin wounds. Further research will examine the relative abundance of paracrine factors present in MSC CM, and examine the effects of human MSC CM on human skin cells.

Keywords: Wound healing,

OP-323

A nanopatterned silk-based composite with sustained release of kartogenin for tissue engineering of articular cartilage superficial zone

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INTRODUCTION: Repair of damaged articular cartilage (AC) is very challenging due to its avascularity, acellularity, and restricted regeneration capacity. Despite technology advancement, remodelling of zonal AC has yet to be adequately addressed which underpins proper cartilage biological and mechanical functions. AC is made of four layers with different ultrastructure and function including superficial zone (SZ). SZ makes up 10-20% of the AC thickness, providing smooth joint movement. Since SZ possess the highest tensile strength in AC, its integrity is vital to overcome the mechanical forces from the articulating surface which is challenging when designing a SZ substitute. Here, by considering the silk high strength and chondro-inductive properties of kartogenin as a drug for MSCs chondrogenesis, we aimed to develop a kartogenin-loaded nanopatterned silk platform mimicking the structure and property of AC-SZ.

METHODS: Nanopatterned silk mesh was made by soft and colloidal lithography followed by oxygen plasma etching and N₂ plasma ion-implantation technique. Then, kartogenin was immobilized on the silk by 3h incubation with 260 nM drug solution at 37 °C. Finally, oligo-chitosan combined with β -GP was casted on the silk surface and incubated at 37 °C for gelation. In cellular studies, the drug release, biocompatibility, and chondrogenic inductiveness of our platform were evaluated with human adipose derived mesenchymal stem cells (hAMSCs). Experiments were performed in triplicates. Data was analysed by t-test/one-way ANOVA where appropriate with p<0.05 considered statistically significant.

RESULTS: The N₂ implantation and nanopillar formation on the silk surface reduced water contact angle and improved the adhesion and proliferation of hAMSCs. Kartogenin was continuously released within 30 days linearly, indicating prolonged steady release. The hAMSCs morphology was altered

within 2 days from elongated to nearly ellipsoidal on highly close-packed nanopillars due to small interpillar distance, enabling cells to develop subsequent focal adhesion contacts in all directions. The nanofeature itself upregulated the expression of chondrogenic genes; COL2A1, SOX9, aggrecan and GAG deposition, all of which were further synergized by concurrent KGN release from the silk. The orientation of type-II collagen expressed by hAMSCs on the nanopillars was parallel to the silk surface similar to that of natural SZ. The nanopatterned silk elastic modulus (921.9 ± 46.2 MPa) and the composite shear modulus (10.2 KPa), are close to those of SZ and synovial fluid, respectively.

DISCUSSION & CONCLUSIONS: Considering the mechanical performance and the in vitro biocompatibility and chondrogenic differentiation results, this novel structure with prolonged kartogenin release could be considered as an alternative for functional engineering of AC-SZ.

Keywords: Cartilage / joint and arthritic conditions, Drug delivery

OP-324

Osteochondral scaffold innovation for repair of large osteochondral defects: from bench to bedside

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INTRODUCTION: The repair of osteochondral defects is one of the major clinical challenges in orthopedics. Well-established osteochondral tissue engineering methods have shown promising results for the early treatment of small defects. However, less success has been achieved for the regeneration of large defects, which is mainly due to the mechanical environment of the joint and the heterogeneous nature of the tissue.

METHODS: In this study, we developed a multi-layered osteochondral scaffold to match the heterogeneous nature of osteochondral tissue by harnessing additive manufacturing technologies and combining the established art laser sintering and material extrusion techniques. The developed scaffold is based on a titanium and polylactic acid matrix-reinforced collagen “sandwich” composite system. The microstructure and mechanical properties of the scaffold were examined and its safety and efficacy in the repair of large osteochondral defects were tested in an ovine condyle model.

RESULTS: The in vivo evaluation period revealed extensive and significantly higher bone in-growth in the multi-layered scaffold compared with the collagen-HAp scaffold, and the achieved stable mechanical fixation provided strong support to the healing of the overlying cartilage, as demonstrated by hyaline-like cartilage formation. The histological examination showed that the regenerated cartilage in the multi-layer scaffold group was superior to that formed in the control group. Chondrogenic genes such as aggrecan and collagen-II were upregulated in the scaffold and were higher than those in the control group. The findings showed the safety and efficacy of the cell-free “translation-ready” osteochondral scaffold, which has the potential to be used in a one-step surgical procedure for the treatment of large osteochondral defects.

DISCUSSION & CONCLUSIONS: It was observed that gross morphological appearance of regenerated cartilage was superior in osteochondral scaffold group compared to the control group. Collagen 2 and Safranin-O stainings confirmed formation of a hyaline-like cartilage. The pQCT examination revealed that the BV/TV ratio in the surrounding subchondral bone was significantly higher ($p=0.01$) in the osteochondral scaffold group (~40%) than that in the control group (~15%). The bone-scaffold contact analysis revealed the bone-implant contact achieved 61%. It is believed that the new bone growth into the Ti matrix at bone section provided a stable mechanical fixation providing a strong support to the overlying cartilage layer leading to an improved cartilage formation.

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Keywords: Cartilage / joint and arthritic conditions, Translation and commercialisation (inc. clinical trials and regulatory approval)

OP-325

An innovative miR-activated scaffold for the delivery of a miR-221 inhibitor to enhance cartilage defect repair

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INTRODUCTION: The development of treatments to restore damaged cartilage that can provide functional recovery with minimal risk of revision surgery remains an unmet clinical need¹. Gene therapy shows increased promise as an innovative solution for enhanced tissue repair^{1,2}. To this end, the delivery of microRNA (miR)-based therapeutics in a sustained and tissue-specific manner offers a promising approach to regulate the fate of specific cell populations by activating or inhibiting entire intracellular pathways². Therefore, this study aimed to develop an innovative advanced miR-activated scaffold biomaterial for enhanced cartilage repair through the delivery of a miR-221 inhibitor using a non-viral vector (cell-penetrating peptide "GET")³ incorporated into a collagen-based scaffold specifically designed for cartilage repair⁴.

METHODS: MiR-activated scaffolds fabrication: composite collagen type I/II-hyaluronic acid (CI/II-HyA) scaffolds were firstly manufactured with a freeze-drying (lyophilization) method as previously described⁴. Then, the GET vector was combined with a miRNA-221 inhibitor to form nano-complexes which were added to the CI/II-HyA scaffolds. In vitro evaluation: miR-activated (MA) scaffolds were cultured using human MSCs in chondrogenic conditions +/- transforming growth factor- β 3 (TGF- β 3) for 28 days.

RESULTS: MA scaffolds +/- TGF- β 3 successfully reduced miR-221 levels in MSCs at day 3, 14 and 28 compared to miR-free (MF) scaffolds demonstrating the capacity to silence miR-221 expression in a sustained and controlled manner up to day 28. Additionally, analysis of the effect of miR-221 silencing on MSC chondrogenic differentiation revealed that MA scaffolds + TGF- β 3 up-regulated aggrecan (ACAN) expression at day 14 compared to MF + TGF- β 3 ($p \leq 0.05$) scaffolds. This result was further supported by improved cell-mediated sulphated glycosaminoglycans deposition/distribution (40.11 $\mu\text{g/ml}$) by day 28 in the MA +/- TGF- β 3 scaffolds.

DISCUSSION & CONCLUSIONS: The results describe an innovative miR-activated scaffold for the delivery of a miR-221 inhibitor in order to enhance chondrogenesis with up-regulated ACAN (a principal cartilage matrix component) expression at day 14 and an enhanced deposition/distribution of cartilage-like matrix in vitro. Taken together, this study thus demonstrates that the incorporation of therapeutic genes into a scaffold designed specifically for cartilage repair might be a promising approach for the treatment of cartilage damage and disease.

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Keywords: Gene therapy, Biomaterials

OP-326

Modeling cartilage pathology in Mucopolysaccharidosis VI using iPSCs reveals early dysregulation of chondrogenic and metabolic gene expression

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INTRODUCTION: Mucopolysaccharidoses type VI (MPS VI) is a severe metabolic disease caused by Arylsulfatase B (ARSB) enzyme deficiency leading to intralysosomal accumulation of glycosaminoglycans (GAGs). MPS VI is a multisystemic disease and progressive and severe cartilage pathology is a common symptom in MPS VI. Although cartilage is one of the major affected tissues in MPS VI patients, the development of cartilage pathology remains poorly understood. In-depth studies to unravel the mechanisms leading to the disease progression in human cartilage tissue affected by MPS VI are required.

METHODS: To develop an in vitro model system to study the cartilage pathology in MPS, we have generated four hiPSC lines from four patient-derived fibroblast. We generated isogenic lines from all hiPSCs using CRISPR-Cas9 in order to control for the high variability between genetic backgrounds of human individuals. Isogenic hiPSCs were differentiated into chondrogenic cells. Polysaccharides (such as GAGs) in enlarged lysosomes were visualized using periodic acid–Schiff (PAS) staining of cytopins. RNA sequencing analysis was used to compare genome wide changes in mRNA expression between chondrogenic cells from all 4 isogenic pairs.

RESULTS: The expression of ARSB was successfully restored by the introduction of a healthy copy into a safe location of the genome. Key chondrogenic genes were expressed after 14 days of differentiation and ARSB RNA expression in chondrogenic cells was 2 to 12-fold increased in gene-corrected cells. Quantification of the number of PAS positive cells showed a reduction in all four gene edited lines to levels comparable to the levels in a healthy control. Genome wide mRNA expression analysis of the isogenic pairs showed the dysregulation of several processes including cell growth and apoptosis, bone and cartilage development, Wnt signaling and metabolic processes.

DISCUSSION & CONCLUSIONS: In this study, we have generated a personalized in vitro disease model for MPS VI with isogenic controls and used this to obtain insight in early stages of disease pathology

in chondrogenic cells. Using the differentiated isogenic pairs, we showed enlarged lysosomes at day 14 of differentiation, indicating accumulation of GAGs. Genome-wide mRNA expression analysis further indicated that chondrogenic cells develop MPS VI-related pathology at an early stage of development and support the conclusion of an early disease phenotype in cartilage. These results indicate that chondrogenic cells display strong disease hallmarks, and sheds novel light on the disease mechanisms involved in MPS VI. This approach may lead to identification of novel targets for therapy to treat the cartilage symptoms in MPS VI.

Keywords: Disease models, Induced pluripotent stem cells

OP-327

Sustained release of locally delivered celecoxib provides pain relief for osteoarthritis: a proof of concept in dog patients

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INTRODUCTION: Joint pain has an intense negative impact on all aspects of quality of life. A common musculoskeletal disease that results in chronic pain is osteoarthritis (OA). OA is a chronic inflammatory and degenerative joint disease often affecting knee and hip joints. Management for joint pain remains ineffective as high doses of oral analgesics (e.g., NSAIDs) only achieve low local drug concentrations and result in adverse effects, while directly injected drugs are rapidly cleared and thus require frequent re-injections. Drug delivery platforms that allow for gradual drug release after intra-articular administration have become of interest as treatment strategy for OA. The aim was to investigate the safety and efficacy of an intra-articular sustained release formulation containing celecoxib (CXB), a COX-2 selective inhibitor.

METHODS: Amino acid-based polyesteramide microspheres (PEAMs), a biodegradable and non-toxic biomaterial platform, were loaded with CXB and employed in two in vivo models of arthritis: an acute inflammatory arthritis model in rats (n=12), and a randomized controlled study in chronic OA dog patients (n=30). In parallel, the bioactivity of sustained release of CXB was evaluated in monolayer cultures of primary dog chondrocytes under inflammatory conditions.

RESULTS: Sustained release of CXB did not alleviate acute arthritis signs in the rat arthritis model, based on pain measurements and synovitis severity. However, in OA dog patients, sustained release of CXB improved limb function as objective parameter of pain and quality of life based on gait analysis and owner questionnaires, respectively. It also decreased pain medication dependency over a 2-month period and caused no adverse effects. Prostaglandin E2 levels, a marker for inflammation, were lower in the synovial fluid of CXB-treated dog OA patients and in CXB-treated cultured dog chondrocytes.

DISCUSSION & CONCLUSIONS: Local sustained release of CXB with CXB-PEAMs is safe and effective to treat chronic OA pain in dogs suffering from natural disease, but was unsuitable to treat acute inflammation in arthritic joints with flares in rats. Follow-up studies with larger sample size, longer follow-up time, and predefined rescue analgesia protocols are needed to investigate whether prolonged local exposure to CXB results in long-term pain reduction, and ultimately, disease modifying effects in dog patients with early OA. Considering the promising data in the current study, this CXB delivery platform could offer a suitable strategy for long-term OA pain management and further advance this concept towards first-in-man studies.

ACKNOWLEDGEMENTS: Thanks Sabine Versteeg, Roelof Maarschalkerweerd, Dick van Zuilen, Nicoline van Klaveren, and Kaat Houben for their help.

Keywords: Cartilage / joint and arthritic conditions, Drug delivery

OP-328

Perichondrocytes of microtia patients as a cell source for cell-based tissue engineering of a human auricle

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INTRODUCTION: Perichondrocytes (PC) from microtia patients might be an autologous cell source for tissue engineering of a human auricle. To generate large constructs like human auricles, high cell numbers are required. Therefore, an extensive in vitro expansion is needed, but little is known about the effects of cell culture on perichondrocytes (PCs). In this study, perichondrocytes from microtia patients were investigated in terms of their stemness properties (migration, colony forming capacity) and surface markers for chondroprogenitor-like cells until passage 4. A gene expression profile was analysed for cells in passage 3 and these cells were cultured in a spheroid model to assess their ability to express cartilage-specific matrix proteins. These results were analysed comparatively with chondrocytes from microtia patients and PC from healthy donors.

METHODS: Perichondrocytes of microtia patients (PC-OM, n=10) and healthy donors (PC-OH, n=10) were isolated by outgrowth culture, chondrocytes from microtic cartilage (CC-M, n=10) by enzymatic digestion. (Ethics vote no: 2018-584N-MA). Colony forming and migration assays were performed to investigate stemness properties. Flow cytometry was used to examine surface markers (CD90, CD44, CD73, CD29 and CD49e). Spheroids (1.5x10⁴ cells/spheroid) were cultured for 42 days in ultra-low attachment plates. Subsequently, the expression of collagen I and collagen II was analysed by immunohistochemical staining. To analyse the gene expression profile the PanPathway-Panel-Kit (770 genes, Nanostring) was used and samples were measured with the nCounter-system (Nanostring). Obtained data were analysed with nSolver 4.0 software (Nanostring).

RESULTS: PC-OM showed comparable stemness properties to CC-M and PC-OH. All selected surface markers were expressed to the same extent on PC-OM (positive cells ranged from 85 to 95%) as on PC-OH and CC-M. The number of positive cells was maintained during cell culture. The gene expression profile from PC-OM is comparable to PC-OH (10 differently expressed genes (deg)) and also to CC-M (40 deg). COL1A (collagen I), COL3A1 (collagen III) were expressed equally in all three cell types while COL2A (collagen II) was not expressed. COMP (cartilage oligomeric matrix protein) was only expressed in PC-OM and PC-OH. In spheroids, collagen I and collagen II were expressed by all cell types.

DISCUSSION & CONCLUSIONS: PC-OM demonstrated chondrogenic properties in cell culture that were comparable to those of healthy donors and chondrocytes from microtic cartilage. In spheroids, PC-OM started to express collagen II, which is important for mechanical properties of hyaline or elastic cartilage. Therefore we conclude that PC-OM are a valuable cell source for cell-based tissue engineering of a human auricle.

Keywords: Stem cells - general, Personalised medicine

OP-329

Thumb Osteoarthritis: Stem Cell Activation, Niche Augmentation and Tissue Regeneration

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INTRODUCTION: Osteoarthritis (OA), of the 1st Carpometacarpal Joint (CMCJ) is a progressive degenerative disease often resulting in Trapeziectomy. Techniques have been used to regenerate cartilage including microfracturing and autologous stem cell transplantation. The effect MF on the Skeletal Stem Cell (SSC) is unknown. Our aims include: 1) Validation of SSC activation 2) Instruction of SSC regeneration 3) Translation of our findings in a xenograft and pig model and ultimately translation towards treatment of 1st CMCJ OA.

METHODS: Animals- Jaxx mice b-Actin Cre-ER, R26Rconfetti, C57BL/6J, NSG. Pigs- Yucatan. Human tissue:

adult femoral heads, foetal phalangeal bones, Human Trapeziums. **METHODS:** FACS, microarray, CFU, EdU, in vitro culture, in vivo models: MF, DMM, renal capsule transplants, parabiosis, xenograft. Statistical analysis-Prism Software: Ordinary one way ANOVA test with Sidak post hoc analysis.

RESULTS: Validated in mouse and human tissue: Acute injury results in a significant ($p < 0.0001$) local increase in tissue-resident SSC and a significant ($p < 0.012$) increase in proliferation of those SSC. SSC intrinsically change in their in vivo differentiation capacity and gene expression following acute injury. Augmentation of the niche with BMP2 and VEGFr1 regenerates stable hyaline-like cartilage confirmed on histology and Atomic Force Microscopy. A large animal model has been established. The presence of SSC in the trapeziums of OA patients has been confirmed.

DISCUSSION & CONCLUSIONS: Regeneration of stable hyaline-like cartilage in vivo is achievable through activation of local tissue-resident SSC through acute injury using MF and can be controlled through augmentation of the niche with growth factors. Our results provide a potential new approach adding to the armamentarium of hand surgeons treating OA of the hand.

ACKNOWLEDGEMENTS: PSF NATIONAL ENDOWMENT FOR PLASTIC SURGERY

BSSH PUMP PRIMING AWARD

BSSH TRAVEL AWARD

Keywords: Stem cells - general, Cartilage / joint and arthritic conditions

OP-330

The effects of low-magnitude pulsed hydrostatic pressure on mesenchymal stem cell motility and ERK-signalling

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INTRODUCTION: We investigated the effect of a single 1-hour mechanostimulation episode on human mesenchymal stem cells using pulsed pneumatic (hydrostatic) pressure to replicate the constitutive biomechanics of the bone marrow microenvironment. Whilst high magnitude forces are widely known to promote osteochondral differentiation and matrix formation in MSCs, the role of physiological forces such as those generated by low-intensity exercise (e.g. walking) are relatively unexplored. We hypothesised that these low-level, constitutive forces play a regulatory role in native stem cell biology which helps maintain the bone marrow niche, and which can be exploited for cell manufacturing.

METHODS: We applied 1 hour of pulsed hydrostatic pressure (100kPa at 1Hz) to hMSCs in well plates using a custom-designed bioreactor as previously described. Cell motility was tracked and quantified using a Phi Instruments Holomonitor, and the MAPK mechanotransduction pathway was investigated using an ELISA. Transcriptomics (RNAseq) of three MSC cell lines was used to identify changes in gene expression 90 minutes after the end of stimulation

RESULTS: Cell migration speed and distance increased two-fold immediately following stimulation, with indications that stimulated cells moved in a more consistent direction (rather than randomly), suggesting an upregulated chemotactic phenotype. ERK signalling in the MAPK pathway at 100kPa was significantly increased over both negative controls (static culture) and positive controls (300kPa pressure). Signalling, reactome and transcription factor analysis using RNAseq data indicated that genes regulated by STAT3 (activated by Janus Kinases, IL-4/13 and MAPK), PPAR γ (activated by ERK), NF κ B and SMAD3 are upregulated by low-intensity mechanostimulation using hydrostatic pressure. Genes involved in integrin-surface interactions and ECM organisation were also differentially regulated.

DISCUSSION & CONCLUSIONS: The biomechanics of the native mesenchymal stem cell niche are surprisingly under-researched, despite the critical importance of mechanotransduction on regulating stem cell fate decisions in bone marrow. We (and others) have previously reported the effects of higher-amplitude forces (>280kPa) on hMSC and bone organoids, but did not identify any effects on cells caused by lower magnitudes of force. In this study we demonstrate that hMSC are highly sensitive to hydrostatic pressure, and low levels of dynamic pressure are shown to play a role in hMSC mobilisation and niche maintenance, in contrast to higher amplitudes which stimulate osteogenic differentiation. The immediate applications of this research are in new engineering strategies for improved stem cell bioprocessing and manufacturing engineered tissues.

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Keywords: Enabling technologies, In vitro microenvironments

OP-331

Role Of Static And Dynamic Mechanical Loading On Cell Fate And Behaviour

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INTRODUCTION: Mechanosensing and mechanotransduction are two mechanisms by which eukaryotic cells perceive and transform mechanical stimuli into biochemical cues [1]. Cell mechanoregulation has attracted a strong interest inasmuch the extracellular and intracellular mechanical forces appear to regulate many aspects of cellular behaviour such as cell shape, cell proliferation, cell polarity and cell differentiation [2]. In particular, the geometric form of cells and their biological functions are inherently correlated. In fact, the cells can discriminate between planar, convex, and concave surfaces [3]. Moreover, there is not only a spatial but also temporal dependence of cell response from its surrounding environment, which has to be analyzed [4].

METHODS: To study the spatio-temporal response of single human adipose derived mesenchymal stem cells, a specific microfluidic platform has been designed. Mechanical cues are applied changing the curvature of substrate to which cells adhere along a preferential direction. To modify curvature in time and space a specific pressure controller is linked to the platform. Moreover, the cell response to curvature variations is observed in real time combining the transfection technique with the confocal microscopy.

RESULTS: The effects of cyclic mechanical stimulation on cell morphology, orientation and focal adhesion organization have been studied. Starting from an undeformed configuration, after 24h of stimulation in static or dynamic conditions, it has been showed a variation of cell orientation and focal adhesion organization depending on the amplitude of curvature and frequency of stimulation. Moreover, stresses and strains developed on the cell-adhered substrate during mechanical stimulation have been quantified with FEM models to directly correlate experimental cell response with their sensed mechanical field.

DISCUSSION & CONCLUSIONS: Due to the correlation between the geometric shape of cells and their biological function, the microfluidic platform was used as a tool to study the cellular spatio-temporal response. The accessibility of this platform guarantees the possibility to view this response in real time. Furthermore, by combining the numerical and experimental approaches, a complete mapping of the stress/strain field developed at the substrate level and sensed by cells can be performed.

ACKNOWLEDGEMENTS: We thank Dr. Velia Siciliano (IIT, Napoli) and her team for the support provided about transfection protocol development.

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Keywords: In vitro microenvironments, Interfaces - engineered

OP-332

Anodized Dental Implants: Custom Nanotopographies towards Tailored Bioactivity

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INTRODUCTION: Conventional titanium-based dental implants require enhanced bioactivity and local therapy for long-term success especially in compromised patient conditions. Poor osseo- and soft-tissue integration and onset of bacterial infection are the leading causes of implant failure. Further, appropriate immunomodulation is also required to initiate implant integration. Can a single implant address these challenges? This research showcases advanced dental implants that have been modified via electrochemical anodization to fabricate controlled nanostructures that enable enhanced osseo- and soft-tissue integration and immunomodulation, while selectively reducing bacterial attachment and biofilm formation.

METHODS: Micro-machined Ti dental implants are anodized to fabricate various titania (TiO₂) nanotopographies, followed by in-depth characterizations evaluating surface topography (SEM and AFM), chemistry (XPS and XRD) and bioactivity relevant features (hydrophilicity and protein adhesion capacity). Next, in separate settings, functions of gingival fibroblasts, osteoblasts, macrophages and oral biofilm are evaluated in vitro, towards studying the influence of nano-engineered implants on soft-tissue integration, osseo-integration, immunomodulation and bacterial attachment, respectively.

RESULTS: Characterization of nano-engineered implants confirmed tailored dual micro-nano features, with enhanced wettability and protein adhesion capacity, as compared to bare implants. Next, selective bioactivity is achieved, whereby a single nano-engineered is capable to maintaining favourable bioactivity of fibroblasts and osteoblasts (with alignment parallel to anisotropic nanotopography), reparative (rather than inflammatory) macrophage proliferation and reduced bacterial attachment.

DISCUSSION & CONCLUSIONS: Ability to tailor responses of specific cells via a simple surface modification is a pioneering attempt. The modified implant with well-defined nanotopography aims to address key challenges associated with long-term success of dental implants, especially in compromised sites.

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Keywords: Biomaterials, Nanomaterials (inc graphene)

OP-333

Biodegradable and electrically responsive polymer hydrogel as a drug delivery system to manage ureteral stent-associated pain

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INTRODUCTION: The increasing interest in personalized pharmacotherapy has prompted the development of sophisticated stimuli-responsive drug delivery systems. Using an external trigger, as an electrical field, enables the spatial-, temporal-, and dosage-controlled release of drugs.¹

Electrically responsive drug delivery systems represent an attractive alternative to treat ureteral stent-associated pain, a frequent side effect of stent placement.² A hybrid approach incorporating a conducting polymer, such as polyaniline, within a polymeric scaffold, such as gelatin, may present advantages for drug delivery application, including electrochemical activity, excellent biocompatibility, and tissue-like mechanical properties.³ Here, we show a biodegradable electroactive hydrogel capable of programmed drug delivery for ureteral applications.

METHODS: Electroactive hydrogels were synthesized by in situ crosslinking of gelatin-graft-polyaniline by genipin, at body temperature. These hydrogels were loaded with ropivacaine, a local anesthetic with proven intravesical effectiveness.⁴ To characterize the hydrogels properly, conductivity analysis, swelling behavior, and degradation assays were performed. The cytotoxicity of hydrogels was assessed following ISO10993-5 and using L929 mouse fibroblast (ATCC NCTC clone 929). In an *ex vivo* porcine assay, hydrogels were placed below a kidney and an abdominal section. Different electrical stimuli (10 and 30 V) were applied for 10 seconds and repeated every 5 minutes between two electrodes placed in the abdominal area. The amount of ropivacaine released from hydrogels, in the presence and the absence of electrical stimuli, was quantified by high performance liquid chromatography.

RESULTS: It was confirmed that hydrogels are semiconductor materials whose conductivity varies proportionally with the amount of polyaniline in the material. The hydrogel composed of 10% gelatin, 30% polyaniline, and 2.5% genipin showed a conductivity of $4,19\text{E-}04 \pm 6,30384\text{E-}05$ S/cm. Due to the appropriate properties of swelling, degradation, and cytotoxicity, the beforementioned formulation was chosen to undergo *ex vivo* assays. Results showed that drug release from hydrogels was higher when electrical stimuli were applied.

DISCUSSION & CONCLUSIONS: We proved that the developed hydrogel system is capable of programmed drug delivery, in response to electrical stimuli. Loaded with an anesthetic with local action, as ropivacaine, these biodegradable electroactive hydrogels may represent a novel suitable strategy for efficient ureteral stent-pain management.

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Keywords: Electroactive materials, Kidney

OP-334

Modular modification of lipid nanoparticles with bioengineered proteins for selective cardiac homing

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INTRODUCTION: Extracellular vesicles have great therapeutic potential in many diseases, including cardiovascular diseases, the leading cause of mortality in developed countries, due to mediated delivery of mRNA and other factors that enhance cardiac repair.¹ Lipid nanoparticles (LNPs) are becoming widely used as extracellular vesicle (EV) mimetics for their application as vectors for the delivery of therapeutic payloads, but they generally lack intrinsic tissue-homing selectivity. Herein, we have designed a novel modular artificial membrane binding protein (AMBP)² platform comprised of two bio-orthogonally reacting protein pairs³ to direct in-house synthesised LNPs to the heart via selective cardiac fibronectin binding.

METHODS: AMBP protein fusions, our LNPs and the constructs resulting from modifying LNPs have been characterised biophysically. The AMBP-modified LNPs have been tested on cells in static and flow experiments, and LNP cardiac homing has been studied in larval zebrafish hearts.

RESULTS: Our modular cardiac-targeting AMBP showed a 20-fold increase in LNP uptake for fibronectin-rich cell types statically, upon no detriment to cell viability, and a 10-fold increase under physiologically relevant shear stress (2 dynes). Additionally, we demonstrated exclusive localization of the cardiac-homing AMBP-modified LNPs in zebrafish hearts, highlighting their therapeutic potential in cardiac disease. Furthermore, we employed mCherry, a red-fluorescent protein, as a payload inside our LNPs to demonstrate only AMBP-modified red LNPs were uptaken by cells.

DISCUSSION & CONCLUSIONS: In conclusion, this constitutes a plug-and-play system that can be readily modified or repurposed depending on the desired therapeutic outcome and can be incorporated in any bilayer membrane.

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Keywords: Cardiovascular, Extracellular vesicles

OP-335

Transfer Tattoo-Like Cell Delivery Platform Using Flexible Thin-Film Transfer

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INTRODUCTION: Cell sheet technology is a promising cell delivery method for tissue engineering and regenerative medicine based on a protease-free cell harvesting and scaffold-free tissue fabrication [1-2]. Here, we introduce a new and facile cell delivery approach combining the advantages of conventional cell sheets and easy handling using a parylene flexible thin-film [3] as a cell transfer carrier.

METHODS: FDA-approved biocompatible parylene-C film was chemical vapor deposited on glass substrates and polystyrene tissue culture plates with thickness of $\approx 3 \mu\text{m}$. Multi-type cells were first cultured on parylene thin films and then transferred to different target surfaces such as 0.6% collagen hydrogel and 2.5% fibrin hydrogel.

RESULTS: We developed a facile cell delivery method using interfacial migration of cells similar to how the transfer tattoo stickers work. Without any additional stimulation, cells could be directly transferred from the cultured parylene surface to another surface with higher cell affinity. Surface optimized parylene provided the intermediate levels of cell adhesiveness for both the stable cell adhesion and the efficient cell transfer. The versatility of this cell delivery process was demonstrated with various cell types, including skin fibroblasts, lung fibroblasts, endothelial cells, osteoblasts, myoblasts, lung epithelial cells and also kidney epithelial cells. Our cell sheet delivery technology included the unique capabilities of 3D multi-layer stacking, freeform trimming and transfer, large-area peeling-off, and 3D curved surface coverage. Finally, multi-layered skin cell sheets were directly transferred to cutaneous wound sites to enhance wound re-epithelialization and re-vascularization in mouse models, demonstrating its therapeutic potential.

DISCUSSION & CONCLUSIONS: This easy-to-use transfer tattoo-like cell delivery platform has wide variety of potential to the therapeutic application of cell sheet technology, to scale-up scaffold-free tissue fabrication, and also to more in-depth studies in cell migration between different planar interfaces.

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Keywords: Cell therapy, Interfaces - engineered

OP-336

Chitosan-Coated Nano-Liposomes as Carriers for Docetaxel Chemotherapy Delivery

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INTRODUCTION: Docetaxel is a chemotherapy drug approved for various cancer types, which is typically delivered through intravenously. As a free drug, the lipophilic docetaxel is associated with poor aqueous solubility and low bioavailability. Drug nano-carriers, such as liposomes, were thus proposed to encapsulate strongly lipophilic drugs to improve the efficiency of their deliveries. Also, many of docetaxel-target cancers (e.g., breast, stomach, head and neck etc.) are housed in a mucus-rich environment, in which drugs can be washed away by body or interstitial fluids easily. As such, polymers with mucoadhesive properties, such as chitosan, were proposed to coat the liposomes (referred as “chitosomes”), for instance, to further enhance the residence time and sustained release of the drug at the site of absorption. Herein, our goal was to investigate the mucosal adherence and cytotoxicity of docetaxel-loaded chitosomes on laryngeal cancer and vocal fold fibroblast cells.

METHODS: Liposomes at a nanoscale were fabricated following our lab’s published protocol. Docetaxel was encapsulated into chitosomes or liposomes as controls. For the mucoadhesive evaluation, docetaxel-loaded chitosomes and liposomes were immersed in mucin 1-contained solutions up to 3 hours. Turbidity analysis was performed via quantitative 500nm absorbance readings. For cytotoxicity studies, two groups of human cells, namely, (I) laryngeal squamous cell carcinomas and (II) vocal fold fibroblasts were exposed to docetaxel-loaded chitosomes, and controls of docetaxel-loaded liposomes or docetaxel alone up to 7 days. LIVE/DEAD fluorescence staining and MTT analyses were performed. Two-way ANOVA tests were used for statistical analyses.

RESULTS: The turbidity of mucin 1-rich solutions from the chitosome group was significantly higher than the controls (~2-fold more cloudiness; $p < 0.05$). LIVE/DEAD images showed a reduction of cancer colonies and fibroblast aggregation after a 7-day treatment exposure in all groups. MTT results further confirmed that upon exposure to docetaxel-loaded chitosomes, about 95% cancer cells (~1-fold more death; $p < 0.05$) and 75% fibroblast cell ($p > 0.05$) became dead at the end of the experiment.

DISCUSSION & CONCLUSIONS: The cationic chitosomes adhered to the mucin particles, resulting in their aggregation and increased turbidity in the solutions. Docetaxel-loaded chitosomes also resulted in more cell death on cancer cells but in lesser degree on healthy fibroblasts. Our in vitro data supported the use of chitosomes for docetaxel encapsulation and mucosal drug delivery for laryngeal cancers.

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Keywords: Cancer, Bioadhesives

OP-337

Polyethyleneimine-decorated gold nanoparticles for simultaneous drug release and enhanced beating properties of hiPSC-derived cardiomyocytes in 3D engineered cardiac tissues

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INTRODUCTION: Cardiovascular diseases caused nearly 19 million deaths in 2020[1] and they annually impose an estimated €200 billion on the European countries. Cardiac tissue engineering is a promising approach to prevent heart failure. However, inducing efficient electrical coupling and incorporating factors to enhance tissue maturation and vascularization remained unsolved. [2] Here, we developed a biohybrid hydrogel to advance beating properties of engineered cardiac tissues and allow concurrent drug release.

METHODS: We synthesized gold nanoparticles (AuNPs) with different sizes (18-241 nm) and surface charges (33.9-55.4 mV) by reducing gold (III) chloride trihydrate using branched polyethyleneimine (bPEI). Nanoparticles were analyzed for their size and charge (dynamic light scattering) and bPEI-AuNPs alone or loaded with drugs were utilized to generate collagen-based hydrogels.

Microstructure and morphology of these hydrogels were studied by scanning electron and optical microscopy. Gelation and mechanical properties of hydrogels were studied by rheology. Cell-containing hydrogels were then studied for cell survival, microstructural changes, gene analysis by qrt-PCR, contractility, and calcium flow.

RESULTS: bPEI-AuNPs permitted normal gelation of collagen. Moreover, they enhanced electrical conductivity of collagen hydrogels from ~40 to 49-68 mS/cm and allowed a slow and steady release of phenylephrine, isoproterenol, and doxorubicin. Neonatal rat and hiPSC-cardiomyocytes survive and exhibit enhanced beating properties in collagen hydrogels containing bPEI-AuNPs. Compared to collagen hydrogels, hiPSC-derived cardiomyocytes exhibited more aligned and wider sarcomeres in bPEI-AuNP-collagen hydrogels. Moreover, presence of these particles resulted in enhanced synchronous calcium flow throughout the tissue. We found an overexpression of connexin-43 without changes in the expression of calcium channels. Phenylephrine-loaded bPEI-AuNPs in collagen resulted in an early onset of synchronous beating of the tissues compared to bPEI-AuNP-collagen hydrogels.

DISCUSSION & CONCLUSIONS: We designed multifunctional electroactive bPEI-AuNPs-collagen hydrogels simultaneously delivering cardiomyocytes and releasing biologically active factors. Owing to the positive charge of bPEI on AuNPs, it is possible to load a variety of negatively charged drugs, peptides, and oligonucleotides. The novel gel represents a versatile platform, is cytocompatible, and promotes the physiological beating rate and contraction amplitude of cardiomyocytes. These constructs can be potentially used for pharmaceutical drug screening or as in vitro produced tissues to treat heart disease.

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Keywords: Electroactive materials, Cardiovascular

OP-338

Hyaluronan functionalized pH-responsive calcium carbonate nanoparticles for local treatment of breast cancer

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INTRODUCTION: Current radio- and chemotherapies are not efficient and many tumors remain resistant to conventional cancer treatments. Specific properties and signaling molecules from tumor microenvironment (TME) have been explored to increase treatment efficacy. An example is the acidic pH of the TME that has been explored to develop stimuli responsive release systems. Herein, we obtained biocompatible calcium carbonate (CaCO_3) nanoparticles that are stable at neutral pH but dissolve at acidic conditions and evaluated their potential as a drug carrier for local cancer treatment.

METHODS: CaCO_3 nanoparticles were produced by co-precipitation of calcium chloride (CaCl_2) and sodium carbonate (Na_2CO_3) in the presence of ethylene glycol [1]. Rhodamine was encapsulated as a model drug. The morphology and diameter of the nanoparticles were determined by scanning electron microscopy and dynamic laser scattering. The particles were coated by layer-by-layer (LbL) assembly of poly-L-lysine and hyaluronic acid (HA). The release was studied in phosphate buffered saline at pH 6.3 and 7.4. Cultures of two breast cancer cell lines (MDA-MB-231 and SK-BR-3) and a healthy epithelial cell line MCF10A (control) were observed under confocal laser scanning microscopy to assess particles internalization and their effect on cell viability (live/dead staining) and metabolic activity (Alamar Blue).

RESULTS: We obtained nanoparticles with a diameter of 560 nm and negative charge that allowed deposition of a LbL coating containing HA - a specific ligand for CD44 receptors overexpressed in cancer cells. The coating and the pH affected the kinetics and quantity of rhodamine release. The coating reduced the release at pH 6.3 two-fold, and three-fold at physiological pH 7.4. Significantly more nanoparticles were internalized after the coating by the cancer cells MDA-MB-231 and SK-BR-3, which inhibited their metabolic activity (48h). The nanoparticles had little effect on the healthy MCF10A cells.

DISCUSSION & CONCLUSIONS: CaCO_3 particles are advantageous encapsulation systems because therapeutics can be conveniently embedded during their production. Our results demonstrated a higher release at the pH found in the TME and a double role of the coating in tuning the release profile and internalization that was dependent on the expression of CD44. Keeping in mind the possibility to tailor the size and morphology of CaCO_3 nanoparticles as well as the thickness of the coating, we suggest that this system is an excellent candidate for local stimuli responsive cancer therapy.

ACKNOWLEDGEMENTS: Portuguese Fundação para a Ciência e Tecnologia (FCT, grants CEECIND/02842/2017, SFRH/BPD/85790/2012; project PTDC/CTM-REF/0022/2020).

REFERENCES: [1] Parakhonskiy et al, J. Nanobiotechnol. 13, 53 (2015).

Keywords: Drug delivery, Cancer

OP-339

Formulation and characterization of hydrogels composed of LXA4-loaded (nano)emulsions: impact of the vector used

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INTRODUCTION: This project focus on the resolution of inflammation which is a key phenomenon in several inflammatory diseases such as pulpitis or periodontitis. Lipoxin A4 (LXA4) is an endogenous anti-inflammatory, pro-resolving molecule that regulates functions of the innate and adaptive immune system. The major challenge in using lipoxins is their hydrophobicity and chemical instability. To overcome these drawbacks, lipoxin was encapsulated using different vectors (emulsion or nanoemulsion) entrapped in silanized hyaluronic acid (si-HA) hydrogels. The first step of this project is to assess the formulation and to characterize these hydrogels composed of LXA4-loaded (nano)emulsions.

METHODS: Lipoxin-loaded emulsions were developed using an emulsifying machine Ultraturrax® (MPE5) or Luer-lock syringes (SE6P). Nanoemulsions (NE) were obtained based on spontaneous nanoemulsification. These vectors were entrapped in 51 kDa si-HA hydrogels with various degree of silanization. Chemical stability of LXA4, encapsulation efficiency and the cumulative release profiles of LXA4 from different gels were studied by mass spectrometry in order to select the best vector. In parallel, the erosion and swelling profiles of hydrogels were studied in phosphate-buffered saline with or without hyaluronidases, in citrate buffer and HEPES at 37°C.

RESULTS: Lipoxin was successfully entrapped in three different vectors composed of 10 µm micelles or 90 nm nanodroplets. Formulations were stables for at least 3 months with an encapsulation efficiency of around 50% for emulsions and 90% for nanoemulsions. The erosion and swelling profiles of the obtained hydrogels were very different: a complete degradation of MPE5 hydrogel (H-MPE5) was observed after 3 days whereas 6 days was required to degrade NE hydrogel (H-NE) and 11 days was needed to degrade SE6P hydrogel (H-SE6P) in hyaluronidases medium. At pH 7.4, only 20% of LXA4 was released from H-MPE5 and H-SE6P after 72 h. Interestingly, in the same conditions 40% of LXA4 was released from H-NE. LXA4 chemical stability results revealed that 77% of LXA4 remained intact after 3 days at pH 7.4. However, a rapid degradation was observed at pH 8.5 and pH 5 showing the crucial role of LXA4 encapsulation.

DISCUSSION & CONCLUSIONS: Nanoemulsions demonstrated the optimal delivery system with the best encapsulation efficiency and drug release profiles. This study highlights the importance of the system used to vectorized LXA4 (emulsion versus nanoemulsion, si-HA hydrogels), to control the molecule release and stability. Moreover, hydrogel features were pH dependent and the system could be adapted function of the intended applications in the perspectives of dental pulp or periodontal regeneration.

Keywords: Biomaterials, Hydrogels and injectable systems

POSTER PRESENTATIONS' ABSTRACTS

PP-001

The fabrication of in-vitro liver tissue microenvironment scaffolds using electrospun polycaprolactone and human ECM

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INTRODUCTION: Liver disease cases have been increasing for several decades and are now responsible for 2 million deaths around the globe [1]. Demand for transplants far exceeds the supply which only satisfies 10% of cases that are on the waiting lists [1]. New methods for the production of in-vitro liver tissue models will help to unlock treatments that can reduce the need for transplantation. Here we investigate further the incorporation of human liver extra-cellular matrix (ECM) into 3D electrospun scaffolds to understand its potential for in-vitro use [2].

METHODS: Human liver samples from five different donors have been decellularised by SDS perfusion leaving the ECM behind. The ECM has then been lyophilised and milled to a powder before incorporating into a solution of polycaprolactone (PCL). The solutions were then electrospun into scaffolds that can support hepatic cells. The ECM and scaffold contents were analysed using Fourier-transform infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and immunohistochemical methods. All fabricated scaffolds were subject to physical and chemical analyses and cultured with an immortalised hepatic cell line (HepG2) or Primary Mouse Hepatocytes (PMHs). The cultured cells on the scaffolds were examined using the following assays including: cell viability, DNA quantitation, immunohistochemistry staining, albumin excretion and gene expression of key functional markers.

RESULTS: The results show that our methods successfully obtain scaffolds containing different human liver ECM samples and allows for comparison between the different donors. Additionally they highlight the biological differences between the immortalised cell line (HepG2) and Primary Mouse Hepatocytes, which are reflected in the observed functional response changes shown upon different scaffold microenvironments.

DISCUSSION & CONCLUSIONS: These studies show that the morphology and composition of electrospun polycaprolactone scaffolds can have a measurable impact on hepatocyte cultures and our methods hold potential for the development of in-vitro hepatic microenvironments.

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Keywords: Biomaterials, Microenvironment and niche engineering

PP-002

Developing a biomimetic, biofunctionalised membrane to promote periodontal ligament regeneration

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INTRODUCTION: Periodontal disease is characterised by progressive gingival inflammation and degradation of the periodontal ligament and alveolar bone which in turn, leads to tooth loss. Guided tissue regeneration (GTR) is a therapy which is used to regenerate the periodontal ligament and alveolar bone. However, the results are not always predictable. The aim of this research was to develop a bio-functionalised, bio-resorbable GTR membrane with enhanced regeneration properties by using a biomimetic coating technique which we have developed to stimulate tissue regeneration [in several skeletal tissues [1].

METHODS: Electrospun, polycaprolactone random-fibre and aligned-fibre membranes were surface-modified with amine groups by cold plasma deposition of allylamine. This process was followed by non-covalent, sequential binding of heparin and pmole amounts of heparin-binding growth factors (TGF β 3, TGF β 1 or FGF-2) to stimulate periodontal tissue regeneration. Scanning electron microscopy (SEM) was used to determine fibre size and morphology of the membranes. Commercially obtained human periodontal ligament cells (HPDLFs) were cultured on the membranes for up to 21 days in the absence of foetal calf serum or added growth factors. Cell viability was assessed using PrestoBlue[®]. Binding and elution profiles of the growth factors (GFs) from the membranes was determined by ELISAs. Total DNA, protein and collagen deposition were determined respectively using PicoGreen[™], bicinchoninic acid, and measuring the hydroxyproline content.

RESULTS: Random and aligned scaffolds (6 mm diameter) were functionalised with 10 μ g of heparin and 10 ng of TGF- β 1, or TGF- β 3 or FGF-2. The biofunctionalised membranes supported cell viability and significantly ($p \leq 0.01$) enhanced cell seeding to the scaffolds. Aligned PCL scaffolds showed a much higher metabolic activity of the seeded HPDLFs than the PCL random scaffolds at all time points tested (days 3,7,21). TGF β 1 and FGF-2 bound more tightly to the membranes than TGF β 3. However, TGF β 3 gave higher levels of biological stimulation of DNA, total protein and collagen deposition.

DISCUSSION & CONCLUSIONS: All GFs supported the attachment and of viability HPDLF cells and significantly ($p \leq 0.01$) stimulated collagen deposition in serum-free medium for at least 4 weeks in the absence of added growth factors. The aligned-fibre membranes produced significantly more DNA, total protein and collagen ($P \leq 0.05$) than random-fibre membranes.

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Keywords: Biomaterials, Biologics and growth factors

PP-003

Creating an artificial intervertebral disc using functionalized silk fibroin and human mesenchymal stromal cells

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INTRODUCTION: Intervertebral disc (IVD) degeneration is the main contributor to chronic low back pain. For this reason, researchers have searched for a suitable biomaterial to repair and/or regenerate the IVD. A promising candidate to fill this gap is silk fibroin (SF), as there have been several approaches in the past to repair either the IVD's nucleus pulposus (NP) or the annulus fibrosus (AF) using SF. However, to date, nobody has attempted to recreate a whole IVD with dimensions similar to that of a human IVD using SF and human mesenchymal stromal cells (hMSCs). Therefore, we aimed to recreate an artificial IVD using NP- and AF- specific SF scaffolds.

METHODS: All scaffolds were made using SF yarn derived from *bombyx mori*. NP scaffolds (diameter: ~15mm, height: ~3mm) were produced based on "fibre-based additive manufacturing" and half of the samples were functionalized with growth/differentiation factor 5 (GDF5). AF scaffolds (diameter: ~35mm, height: ~10mm) and patches (8mm x 8mm) were manufactured as an embroidered yarn fabric designed to morphologically mimic AF tissue. Bone marrow-derived hMSCs were seeded at a density of 1,333 cells/mm³ onto the NP scaffolds and 3,000 cells/mm³ onto the AF scaffolds, respectively, which is considered to be one-third the cell density found in human NP and AF tissue. The scaffolds were first cultured with FBS containing medium and after three days (Day 0), it was exchanged for chondrogenic inductive medium for another 21 days. Downstream analysis included the measurement of the cell activity, DNA content, glycosaminoglycan (GAG)/DNA ratio, histology, and gene expression.

RESULTS: The data revealed that the cell activity was the highest at Day 0 for NP scaffolds (~600 RFU/μg DNA) and then decreased and stabilized at half of the initial value. Contrary, the AF samples' activity remained stable (~520 RFU/μg DNA). Although the cells proliferated on the scaffolds, the GAG/DNA decreased after seven days and stabilized at a modest value (~1.7 μg/μg). Interestingly, histological analysis revealed a gradual increase of GAG over 21 days, with the highest amount found in the GDF5-functionalized NP scaffolds. Finally, qPCR showed an upregulation of *aggrecan* (up to 83-fold), *collagen type II* (up to 885,000-fold), and *CD24* (up to 478-fold) at Day 21.

DISCUSSION & CONCLUSIONS: Although these data are preliminary, the study showed a novel approach to create an artificial IVD using NP- and AF-specific scaffolds made of SF with the goal to regenerate an IVD.

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Keywords: Differentiation, Stem cells - general

PP-004

Combinatorial analysis of tag peptides for enhancement of cell-adhesion short peptides

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INTRODUCTION: For higher bio-compatibility of medical implants, functionalization of their surface to enhance their regenerative performance, balancing the objective reaction enhancement and the unwanted reaction reduction, is an effective approach. Especially with the medical implants for circulatory system, surface modification and its effect have critical life-threatening effect.

Our group have been reporting short functional peptide peptides (3-9) with cell-selective adhesion [1] to support such regenerative balance in the implanted region. Compared to large proteins extracted from animal-derived tissues, short peptides can offer higher purity and productivity by their chemical synthesis. However, one of the disadvantages in short peptides are the difficulty in immobilizing them with their original sequence potency. The length or methods of immobilization can critically decrease their function. Our group had been working on finding good combinational match between the short peptides and its immobilized support, and have found that closely neighboring physicochemical property control is important [2]. We here report our comprehensive combinational effect of short tag peptides on the target short functional peptides, the cell-adhesion peptides, on the peptide array system.

METHODS: For peptide analysis, peptide and tag peptide conjugated library was designed by SPOT peptide array technique on cellulose membrane by conventional Fmoc chemistry. The target peptides' bio-function was assayed directly on the peptide array. We compared several cells on the same peptide-tag combination, and comprehensively compared the effects of short peptide sequences.

RESULTS: From the combination study, it was found that there were critically effective peptide function enhancement effect even by single amino acid, and such combinational importance was confirmed for controlling cell adhesion.

DISCUSSION & CONCLUSIONS: Our result indicated that the immobilization of short functional peptides is critically affected by their neighboring sequences. This result leads to a simple and cost-effective surface peptide functionalization concept for better biocompatible medical implant surfaces.

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REFERENCES: [1] *Biotechnol Bioeng.* 2012 Jul;109(7):1808-16.

[2] *Anal Sci.* 2016 32(11):1195-1202.

Keywords: Biomaterials, Enabling technologies

PP-005

Development of tissue adhesion barrier sheet functionalized with cell-selective adhesion peptides

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INTRODUCTION: It is known that adhesions in the surrounding tissue frequently occur in the process of wound healing after surgery. Adhesions after surgery rises risks in organ dysfunction and can trigger life-threatening troubles in the second surgery. The formation of such tissue adhesions is a part of the repair mechanism; therefore, it is difficult to simply inhibit them by drug administration. Medical devices play an important role to rescue such tissue adhesion formation as a supportive device or with itself. Anti-adhesion sheets are one of the effective medical devices used in thoracic surgeries. However, conventional polymers used in the present anti-adhesion sheets have low biocompatibility or regenerative performance.

Our research group has been reporting c functional peptides to provide the biomaterial surface “cell-selective performances”, such as cell adhesion or proliferation to enhance the regeneration of implanted area with the combination of reducing negative effects triggered by unwanted cells [1]. In this study, we report the effect of cell-selective peptides to functionalize the polymer surfaces for anti-adhesion medical devices.

METHODS: Cell-selective adhesion peptides for controlling anti-adhesion effect were screened by SPOT peptide array technique which is synthesized on cellulose membrane by conventional Fmoc chemistry [2]. The cell-selective effects were analyzed directly on the SPOT peptide array or on the objective polymers. After 2 hours of cell adhesion, the adhesive cells were measured by fluorescence plate reader in high throughput manner.

RESULTS: As a result, we found several new sequences and their common physicochemical properties to enhance the adhesion of objective cells together with their inhibiting effect on un-wanted cells. The relative adhesion of objective and unwanted cells on our new peptide indicated that we can form the cell-selective peptide functionalized surface on medical device composing polymers.

DISCUSSION & CONCLUSIONS: Our functional peptides to function as cell-selective surface functionalization peptides was confirmed to preserve their function on practically used medical device polymers. The peptides property rules and its surface modification methods should contribute to new biopolymer functionalization for anti-adhesion medical implants.

ACKNOWLEDGEMENTS: This work is partially supported by JSPS KAKENHI Grant Number 16K15630, 18K14061, 19H03737.

REFERENCES: [1] Biotechnol Bioeng. 2012 Jul;109(7):1808-16. [2] Tetrahedron. 1992 48(42):9217-32.

Keywords: Interfaces - biological, Composite materials

PP-006

Enhancing Endothelial Colony Forming Cell Recruitment by Peptide-Biofunctionalized Cardiovascular stents

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INTRODUCTION: Diabetes is associated with increased risk of late stent thrombosis following percutaneous coronary intervention, which could be caused by the delayed endothelialization of the stent lumen. The endothelium plays a key role in hemostasis, and thus, restoring a functional endothelium could protect from stent thrombosis. Biofunctionalization using specific molecules to induce endothelialization can prevent thrombosis. Blood endothelial progenitor cells (EPCs) represent a potential source for in situ-endothelialization of vascular stents. In this study, we biofunctionalized bare metal stents (BMS) using the EPCs specific peptide (TPSLEQRTVYAK)(1). **METHODS:** Cobalt chromium stents were dip-coated with polycaprolactone (5%), followed by activation using 20% ethyldiamine at 40°C. Samples were incubated with a heterobifunctional NHS-polyethyleneglycol-maleimide crosslinker (NHS-PEG-Mal, 1mg/ml) for 2-3hrs at room temperature, followed by an incubation with TPSLEQRTVYAKGGGSC. FITC-tagged peptide was used to confirm peptide immobilization. Endothelialization was tested using blood outgrowth endothelial cells (BOECs) isolated from type 2 diabetes patients. Specificity of the modification was tested using Human coronary artery smooth muscle cells (HCASMCs) and HUVECs.

RESULTS: Immobilization of FITC-tagged peptide on stents resulted in a homogenous fluorescent signal indicating successful modification. Proliferation of BOECs was increased on modified stents when compared to untreated and NHS/Maleimide controls following 4 and 7 days of culture (AlamarBlue assay; OD 570-620 for UT, NHS/Maleimide and TPS stents at day 4 were: 0.18 ± 0.03 , 0.11 ± 0.02 , and 0.29 ± 0.01 respectively, $p=0.0002$; ODs at day 7 were: 0.13 ± 0.03 , 0.09 ± 0.03 and 0.25 ± 0.02 respectively, $p=0.02$; $n=4$, ANOVA). Staining with CD31, VEGFR2 and phalloidin showed an enhanced coverage of the modified stents with BOECs when compared to untreated stents. Capture assays of HUVECs and HCASMCs revealed a reduction in cell adhesion to stents modified with NHS/Maleimide alone and with TPS peptide.

DISCUSSION & CONCLUSIONS: This study aimed to enhance stent endothelialization to reduce the incidence of in-stent thrombosis in diabetic patients. Biofunctionalization of PCL-coated BMS with the EPC specific peptide TPSLEQRTVYAKGGGSC through covalent crosslinking resulted in induced proliferation of diabetic BOECs and full coverage of the stents by day 14 of culture. The use of crosslinker alone suppressed the adhesion of cells due to the PEG arm in the crosslinker. Studies are ongoing to validate stents interaction with platelets.

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Keywords: Cardiovascular, Biomaterials

PP-007

Tenogenesis after equine tendon-derived extracellular matrix bioprinting with adipose tissue mesenchymal stem cells

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INTRODUCTION: The many barriers of tendon regeneration demand the developing of innovative bioengineering techniques. The association of extracellular matrix (ECM) scaffolds with cells capable of tenogenic differentiation in a bioprinted material is a promising strategy that aims to reach good results in tendon repair. The aim of this study was to evaluate an ECM from equine tendon associated with adipose tissue-derived mesenchymal stem cells (adMSCs).

METHODS: This study was conducted according to the Ethics and Animal Use Committee approved under the protocol number 0194/2019.

Constructs were printed by direct extrusion, using 1x10⁶ ad-MSCs/mL. Cell viability was assessed at time points 1, 7 and 14 days of culture. After this period, constructs were divided in two groups: the first group (GTensile) had the vertices of the constructs anchored over a polydimethylsiloxane (PDMS) surface, generating a resistance to cell contraction, while the constructs of the second group (GFree) were maintained loose in petri dishes.

Cell viability was evaluated by calcein and propidium iodide at 24h, 7 and 14 days of culture, whereas gene expression was assessed initially and after 21 days. Evaluation of the constructs was performed 2h and 21 days after bioprinting and included structural analysis, as well as immunohistochemistry for collagen fibril organization and synthesis of collagens types I and III, and gene expression of ECM synthesis and tenogenic biomarkers by rt-qPCR.

Biocompatibility was assessed by construct implantation in vivo.

RESULTS: Synthesis of types 1 and 3 collagen was observed by immunohistochemistry. Cells were viable through time and GTensile expressed Mohawk, Tenascin C, Decorin, Byglican, Collagen 1 and Collagen 3 genes, and ECM proteins.

DISCUSSION & CONCLUSIONS: Although tenogenesis and scaffold organization were observed in both groups, an overall more organized aspect was perceived in GTensile, with more fibrill parallelism and better scaffold structure. Cell contraction against the anchored vertices of GTensile constructs provided tensile strength capable of signalling a more adequate tenogenesis. Our results indicate that the association of an ECM bioink and adMSCs induces the tenogenic commitment of adMSCs through cell signalling, presenting both in vitro and in vivo biocompatibility that places this new biomaterial as a potential candidate for innovative techniques of tissue engineering.

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Keywords: Biomaterials, Cell therapy

PP-008

Lauryl hyaluronan derivative for effective treatment of dry eye syndrome

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INTRODUCTION: The global prevalence of dry eye syndrome was estimated to range from 5 to 50 %, depending on various parameters. Dry eye syndrome can be described as conditions when functional tear film cannot be produced physiologically. This malfunction influences the ocular surface and can lead to the symptoms such as visual discomfort, decreased vision, corneal damage, and pain. It considerably affects the quality of life and works productivity. Artificial tears based on solutions of suitable polymer are considered an effective and least invasive treatment. The used polymer should have good biocompatibility and mucoadhesion. Artificial tears containing hyaluronan showed acute and long-term therapeutic benefits such as improved ocular surface function, corneal epithelium healing, and protection of goblet cells. However, hyaluronan has rapid clearance and limited mucoadhesion. Furthermore, there is still not enough data relating the physicochemical properties of polymer solutions with their biological effects applicable to dry eye treatment. [1-3]

METHODS: Hyaluronan was grafted by lauryl groups to improve its physicochemical properties while keeping excellent biocompatibility. The water solubility and other properties depended on molecular weight and degree of substitution, which were determined by size-exclusion and gas chromatography. [4]

RESULTS: Hydrophobic interactions between lauryl side groups changed molecular dynamics as was determined by rheology. The chain stiffness decreased while intermolecular interactions strengthened. It led to a significant decrease in the crossover concentration between unentangled and entangled dynamics. The lauryl derivative had higher viscosity and steeper concentration dependence above the crossover concentration compared to native hyaluronan. The mucoadhesive index determined from rheology increased with molecular weight and was several times higher for lauryl derivatives than native hyaluronan. The application's relevant biological properties were determined by in-vitro protection of human keratinocytes against dehydration.

DISCUSSION & CONCLUSIONS: Improved physicochemical properties and enhanced interactions with mucin of lauryl derivative led to increased cell protection compared to native hyaluronan. Concentration and molecular weight had a similar influence on biological and physicochemical properties. It allowed using rheological results to design effective formulations. Significantly lower concentrations of lauryl derivative were sufficient to achieve the same biological effect as native hyaluronan. It demonstrated the perspective of amphiphilic hyaluronan derivatives for ophthalmic applications.

ACKNOWLEDGEMENTS: TACR TREND project FW01010060 is greatly acknowledged.

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Keywords: Eye, Polymers - natural / synthetic / responsive

PP-009

Engineering immunomodulatory materials for regenerative medicine

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INTRODUCTION: Immunomodulatory biomaterials can modulate the host immune response and lead to successful outcomes in a variety of regenerative applications, however, there is still a lack of understanding of biomaterial-immune system interactions. In addition, the endotoxin levels of biomaterials are rarely considered, despite endotoxin significantly affecting the immune response and thus the performance of biomaterial-based approaches. Collagen is widely utilised in tissue engineering and regenerative medicine, with mammalian collagen (type I) mainly used. However, the immunomodulatory and regenerative potential of jellyfish collagen ("type 0") derived from *Rhizostoma pulmo* as an alternative to mammalian collagen was investigated. Jellyfish collagen is sustainable and disease vector free, avoiding the ethical/safety concerns associated with mammalian collagen use. Furthermore, jellyfish collagen has been shown to trigger tissue repair as opposed to excessive inflammation, dictated by the balance of M1 and M2 macrophages, which has been demonstrated in models for wound healing and bone regeneration.

METHODS: Therefore, the endotoxin content of jellyfish collagen was assessed using the limulus amoebocyte lysate (LAL) assay and compared with mammalian alternatives. The production of TNF- α , a strong pro-inflammatory cytokine, by peripheral blood mononuclear cells (PBMCs) was then measured following culture with jellyfish collagen for 24 hours. Finally, the effects of encapsulating human mesenchymal stem cells (MSCs) in jellyfish collagen hydrogels on cellular morphology and viability (by immunostaining) and metabolic activity (by PrestoBlue) after 1, 4, and 7 days of encapsulation was assessed.

RESULTS: The endotoxin levels of jellyfish collagen were above regulatory limits (>0.5 endotoxin units (EU)/ml), while the endotoxin levels of mammalian (bovine and rat tail) collagens were below regulatory limits (<0.5 EU/ml). Jellyfish collagen also induced a high production of TNF- α from PBMCs, suggesting that a strong inflammatory response would be elicited, although this may be due to the presence of endotoxin. The morphology, viability, and metabolic activity of MSCs did not appear to significantly differ following 7 days of encapsulation, however, the rapid degradation of the hydrogels may have affected these results.

DISCUSSION & CONCLUSIONS: Thus, despite jellyfish collagen demonstrating promise as an alternative to mammalian collagen, the high endotoxin levels (and high production of TNF- α by PBMCs), as well the unstable nature of jellyfish collagen hydrogels currently render them unsuitable for use in vivo. The endotoxin levels of jellyfish collagen must be reduced below regulatory limits, and the stability of jellyfish collagen hydrogels must be increased to effectively investigate the potential of jellyfish collagen for use in tissue engineering and regenerative medicine.

Keywords: Immunity / immunomodulation / macrophage, Hydrogels and injectable systems

PP-010

The Effect of Crosslinking Conditions and Poly(ethylene) Oxide (PEO) addition on GelMA Properties and Bone Forming Ability

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INTRODUCTION: Hydrogels have been recently used to construct templates guiding bone regeneration by promoting the differentiation of cells into bone tissue. Although their stiffness is insufficient for obtaining mineralized and "ready-to-use" bone, they might provide an easy tunable platform for unmineralized new bone formation (osteoid). Osteoid properties differ from mature bone and should be carefully studied to establish a platform that mimics bone reorganization all along. Thus, this work aims at tuning the physical properties of GelMA hydrogels through microarchitecture to support bone mesenchymal stem cells (hBMSCs) proliferation and bone tissue formation.

METHODS: To resemble the osteoid stiffness, two different crosslinking methods of GelMA (5 and 10% w/v) with 0.1% LAP addition were proposed: standard-crosslinking (SC) using visible light (VL) and dual-crosslinking (DC) consisting of physical gelation and VL. GelMA-10% served as a control and was optimized initially (G10-SC); GelMA-5% underwent DC (G5-DC). To obtain a more hierarchical pore structure, 20 vol% of PEO (~300 kDa) was also introduced into G10-SC and G5-DC. Dynamic mechanical analysis was performed to assess the stiffness of the constructs. To evaluate the physical gelation of G5 on hBMSCs, the percentage of reduction of AlamarBlue was calculated. The embedded cells were cultivated in the differentiation medium up to day 21 (D21). The evaluation of hBMSCs included live/dead staining followed by cell morphology analysis by immunofluorescence. To select the GelMA microarchitecture supporting bone tissue formation, the alkaline phosphatase (ALP) production normalized toward DNA content was also detected.

RESULTS: The physical gelation of G5-DC at 5 °C led to obtaining similar stiffness to G10-SC (~18 kPa) with reduced time of chemical crosslinking (12s vs. 60s). One-hour incubation of G5-DC did not significantly affect cell metabolism in comparison to G10-SC. The normalized ALP activity in G5-DC was ~3 and 1.5 times higher than in G10-SC at D7 and D14, respectively, whereas its DNA content was ~3 times lower. The PEO addition did not enhance the ALP production and, during its gradual removal, cells were entrapped in the hydrogel structure, depicting roundish morphology, especially in G10-SC.

DISCUSSION & CONCLUSIONS: We have demonstrated that by applying the physical gelation of GelMA prior to its chemical crosslinking, improved mechanical properties of hydrogel can be obtained without hampering cells' survival. The data suggest that G5-DC is a good candidate for bone tissue applications, promoting ALP production and maintaining its structure over the incubation period.

ACKNOWLEDGEMENTS: This research was supported by grant no. UMO-2021/41/N/ST5/04220 from the Polish National Science Centre.

Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-011

Permeability test bench for characterizing tissue engineering scaffolds

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INTRODUCTION: The ability of tissue engineering scaffolds to be permeated by fluids (permeability) strongly influence their suitability to be colonized by cells. Thus, characterizing scaffolds permeability could provide crucial information in view of their applications¹. Several methods have been proposed for assessing scaffold permeability, however, defined protocols are still missing and, in case of soft scaffolds, their deformability pose challenging tasks². Here, a versatile permeability test bench (PTB) for characterizing hard and soft scaffolds was developed and preliminarily validated by testing hard scaffolds and comparing the results with a previously validated reference test bench (RTB)³.

METHODS: The PTB is based on the pump method¹. The permeability chamber (PC), equipped with custom gaskets and grids, allows housing cylindrical samples (height=1-14 mm, diameter=8-27 mm). The PC is part of a closed-loop hydraulic circuit composed of a reservoir, a peristaltic pump, silicone tubing, and two pressure sensors (HJK) upstream and downstream the PC. Demineralized water is used. Pressure measurements are acquired by a DAQ (National Instruments) using a custom LabView Interface. Upon imposing a defined flow rate (guaranteeing laminarity), permeability (k) is calculated using the Darcy model:

$$\Delta P/L = \mu/k \cdot (Q/A)$$

where ΔP is the pressure drop across the sample, L the sample thickness, μ the viscosity, A the cross-section area, and Q the flow rate. For PTB validation, 3D-printed PLA scaffolds with different geometries and commercial bone scaffolds (SmartBone) were tested at 5 mL/min ($n=4$ for each scaffold) and the results were compared with those obtained using the RTB³.

RESULTS: Performance tests confirmed watertightness and functionality of the PTB. The permeability values of PLA scaffolds tested within the PTB and the RTB were respectively: $4.1 \cdot 10^{-10} \pm 3.1 \cdot 10^{-10} \text{ m}^2$ and $4.0 \cdot 10^{-10} \pm 5.5 \cdot 10^{-11} \text{ m}^2$ for perpendicular geometry, $2.4 \cdot 10^{-10} \pm 1.7 \cdot 10^{-10} \text{ m}^2$ and $2.8 \cdot 10^{-10} \pm 5.2 \cdot 10^{-11} \text{ m}^2$ for oblique geometry, $2.4 \cdot 10^{-10} \pm 1.4 \cdot 10^{-10} \text{ m}^2$ and $2.2 \cdot 10^{-10} \pm 4.0 \cdot 10^{-11} \text{ m}^2$ for random geometry. The permeability values of SmartBone scaffolds were $2.9 \cdot 10^{-10} \pm 2.6 \cdot 10^{-10} \text{ m}^2$ (PTB) and $2.7 \cdot 10^{-10} \pm 3.1 \cdot 10^{-11} \text{ m}^2$ (RTB). The normalized errors between PTB and RTB permeability values were less than 1.

DISCUSSION & CONCLUSIONS: A versatile test bench for characterizing the permeability of tissue engineering scaffolds was developed and validated for hard scaffolds. Although the PTB measurements exhibited a higher dispersion, the measured permeability was compatible with that obtained using a reference setup. Optimization of the PTB data acquisition system is in progress for reducing measurement uncertainty and tests using soft scaffolds are ongoing.

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²Podichetty et al., J Biomed Mater Res, 2014.

³Schiavi et al., Meas Sci Technol, 2012.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Additive manufacturing

PP-012

Development of a biosynthetic corneal endothelial grafts to alleviate donor corneal shortage

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INTRODUCTION: In corneal endothelial diseases such as Fuchs' endothelial dystrophy (FED) endothelial cells are dysfunctional and when they reach a critically low level, fluid accumulates in the corneal stroma (oedema), causing cloudiness, corneal thickening, and blurred vision. FED is currently treated with a corneal transplant that typically uses cadaveric tissue in a 1 to 1 ratio of donor to recipient. Our aim was to design a biosynthetic graft that combines a poly-epsilon-lysine (PεK) based hydrogel with expanded corneal endothelial cells (CECs) to increase the number of patients we can treat by producing more than 20 implantable grafts per donor cornea.

METHODS: The peptide PεK, was modified by covalent functionalisation with methacrylamide residues as a simple synthetic strategy to achieve customisable, noncytotoxic UV crosslinked hydrogel networks. This allows more flexibility in the creation of hydrogels of <100µm. The PεKMA hydrogels were evaluated mechanically by compression, stress relaxation for viscoelasticity, and transparency for use as a corneal graft. Human CECs expanded from donor eyes were seeded 1800/mm² onto PεKMA hydrogels to investigate cell compatibility and endothelial cell monolayer formation over 7 days. Cells were characterised immunochemically using typical endothelial markers such as ZO-1 and Na⁺K⁺ATPase.

RESULTS: We have produced a hydrogel with a compressive peak stress of 0.055 MPa and a transparency of over 90% in the visual light spectrum. The hydrogels can be loaded into a clinical delivery device such as an Endoserter or Geuder Szurman single-use DMEK-cartridge and released into a porcine anterior chamber model without damage. The chosen hydrogel allows growth and maintenance of a monolayer of human CECs, with cells showing typical cobblestone morphology and expression of ZO-1 at the junctions.

DISCUSSION & CONCLUSIONS: We have demonstrated that a PεKMA hydrogel can be manufactured with appropriate properties for surgical manipulation and transparency as a biosynthetic corneal graft, which also allows expansion of CECs from one donor to produce multiple implantable grafts. The graft could be used as a surgical therapy to treat endothelial related eye diseases aiming to decrease the current long waiting times for patients.

ACKNOWLEDGEMENTS: -

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Keywords: Eye, Biomaterials

PP-013

Application of silk as anticorrosion and antibacterial film on AZ31 magnesium alloy

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INTRODUCTION: Magnesium alloys, due to their unique properties such as intrinsic antibacterial behaviour, similar density to human bone, natural biodegradability, etc. can be optimal option for orthopaedic applications. However, poor corrosion resistance of Mg alloys is the main hurdle for their practical application. Here, the surface of AZ31 alloy was coated with silk to be protected from corrosion in simulated body fluid (SBF) and biomimicked nanocones were introduced to the surface of silk film to enhance the antibacterial properties.

METHODS: Silk cocoons were boiled for 30 minutes in water solution with 0.02 M Na₂CO₃ and washed afterward, followed by dissolving in 9.3M LiBr solution and then dialyzed for 48 h. AZ31 substrates were spin-coated with silk solution and nanocones were fabricated on the surface of silk film via colloidal lithography and oxygen-plasma etching. SEM, FTIR, Raman spectroscopy, AFM, electrochemical impedance spectroscopy, potentiodynamic polarization and adhesion tests were done to characterize the platform. Agar plate counting and live/dead staining methods were used to assess antibacterial properties of samples against E. coli and S. aureus bacteria strains while live/dead staining of MC3T3 cells was used to show biocompatibility of the platform.

RESULTS: Nanopatterns with 400 and 300 nm, height and centre-to-centre distance, respectively, was fabricated on the silk film. This film increases corrosion properties significantly (near to 104 times) by decreasing corrosion current from 79μA/cm² to 0.01μA/cm² and increasing the corrosion potential from -1.58V to -0.78V. The immersion test results showed that weight loss of Mg alloy was decreased around 50% after 1 day and the similar trend was seen for Mg ions leaching. β-sheets in silk, which are responsible for the good corrosion behaviour, started to deteriorate after 1 day, detected by FTIR and Raman spectroscopy. Antibacterial properties of Mg alloys stemmed from releasing Mg ions and by improving in corrosion properties of the alloy, there would be a loss in its antibacterial properties. Here, nanocones compensated this lack and in overall, enhance both corrosion and antibacterial properties. Also, by decreasing the amount of leached Mg ions and consequently pH of medium, cytotoxicity of platform was decreased and less cells were died due to high alkalinity of the medium.

DISCUSSION & CONCLUSIONS: Introducing nanopatterned silk film as a coating on the surface of AZ31 magnesium alloy, significantly enhanced its corrosion properties while successfully counteract the loss of inherent antibacterial characteristics of the AZ31 due to lesser Mg ions leaching.

Keywords: Biomaterials, Polymers - natural / synthetic / responsive

PP-014

GELMA-PVP/MBGs COMPOSITES FOR BONE TISSUE ENGINEERING

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INTRODUCTION: One of the main objectives of tissue engineering (TE) is to obtain biocompatible materials capable of regenerating body tissues. Since both gelatin and PVP are highly biocompatible and non-toxic, the mixture of these two polymers is interesting to prepare a functional polymer composite [1]. This study proposes a biomaterial composed of methacrylated gelatin (GelMA), polyvinyl pyrrolidone (PVP) and bioactive mesoporous glasses (MBG) synthesized by photo initiated radical polymerization [2].

METHODS: Methacrylated gelatines (GELMA(w/v)) (1-16.7) were synthesized. MBG was synthesized by the sol-gel method combined with the EISA method with molar composition of 85%SiO₂-10%CaO-5%P₂O₅. GELMAs were mixed with 1-vinyl-2-pyrrolidone (PVP). Photoinitiator-I2959 was added and was poured into a 2 cm teflon-mold and introduced into UV-lamp. They were characterized by XRD, N₂ adsorption, FTIR, SEM microscopy and swelling assay. In vitro cytocompatibility assays were carried out with MC3T3-E1 cells.

RESULTS: Nitrogen isotherms on MBG indicate of 6.5 nm pores, 288 m²/g surface area. FTIR spectra of GelMA samples with different degrees of methacrylation present a band at 3278 cm⁻¹ related to the presence of the peptide bond (N-H vibration) of amide A, as well as the signal at 3070 cm⁻¹ attributed to the vibration of the C-H bond of amide in all the spectra. Swelling results reflected all samples a high percentage of swelling. Only GELMA obtained better cell proliferation, being higher in the most cross-linked since it is more stable. The most methacrylated gelatin/MBG presented good proliferation, so addition of MBG is beneficial for the cellular development. The containing PVP had significantly lower cell proliferation results compared to those without PVP. Cell differentiation was detected with the presence of calcium deposits on the membranes by Alizarin red staining by measuring the absorbance of mineralization. All materials present good cell differentiation, being higher than the control. Specifically, MBGs favour osteoblastic differentiation because they are highly biocompatible and bioactive materials, being higher in the materials containing the most methacrylated gelatin.

DISCUSSION & CONCLUSIONS: The combination of GELMA/PVP/MBG were synthesised and confirmed their physicochemical properties such as microstructure, porosity and swelling. Their cytocompatibility and osteogenic potential were characterized by proliferation and differentiation assays on preosteoblastic cells. The biomaterials obtained with higher degree of methacrylation and of MBG showed good cytocompatibility.

ACKNOWLEDGEMENTS: Instituto de Salud Carlos III (PI20/01384), ERC-2015-AdG Advanced Grant Verdi No.694160.

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Keywords: Composite materials, Bone and bone disorders (osteoporosis etc)

PP-015

The Influence of Nano and Micro Scale Surface Topography on Cell Migration

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INTRODUCTION: Nanofibres can be produced via electrospinning from a range of natural and synthetic polymers, and can share many characteristics of the *in vitro* extracellular matrix (ECM) environment. For this reason, electrospun nanofibres are an exciting area of research in biomaterial scaffolds for tissue engineering.

It has been established that different cell types respond differently to topographies in the nano- and micro- scale. Therefore, we have been investigating the effects of combining the nanoscale topography of nanofibres with microscale topography that has been imprinted onto a sheet of electrospun nanofibres.

METHODS: Polycaprolactone (PCL) nanofibres were fabricated from a 10% w/v solution, using a 50:50 chloroform: dimethylformamide solvent system. A 20 kV voltage was applied to a 21 gauge needle, which the solution was fed through. A grounded rotating mandrel was used to collect highly aligned nanofibres.

A series of 3D printed stamps were produced, with aligned grooves ranging from 25 μm to 250 μm , and used to imprint micro scale grooves onto the nanofibre sheets.

RESULTS: Using the methods described above, we were able to produce nanofibres with diameters as low as 200 nm, that were 77% aligned along the dominant fibre direction. We were then able to imprint micro- scale grooves onto these sheets without significantly altering the fibre diameter or morphology.

We will present data describing the effect of different combinations of aligned nanofibres and these micro- scale grooves on fibroblast morphology, mobility and scaffold infiltration.

DISCUSSION & CONCLUSIONS: Given the previously published data describing different cell behaviour when cultured on nano- and micro- patterned surfaces, combining both nano- and micro- scale topographies could be used in tissue engineered scaffolds to direct cell behaviour and encourage tissue regeneration *in vitro*. This could be particularly useful in tissues with low regenerative capacity, such as oesophageal tissue.

ACKNOWLEDGEMENTS:

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Keywords: Polymers - natural / synthetic / responsive, Fibre technology

PP-016

Production of porous polycaprolactone polyHIPEs as substrates for *in vitro* 3D breast cancer cell culture

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INTRODUCTION: Cancer is becoming a huge social and economic burden on society, being the most significant barrier to life expectancy in the 21st century. In particular, breast cancer is one of the leading causes of death for women. One of the most significant difficulties to finding efficient therapies for specific cancers, such as breast cancer, is the efficiency and ease of drug development and testing. Micro-physiological systems are under development to mimic the structural and biological complexity of human tissue, thus, becoming increasingly popular as an alternative to animal testing for pharmaceuticals.¹ However, the lack of a system for perfusion within current models that could mimic the vasculature of normal tissues remains a challenge.

METHODS: High molecular weight polycaprolactone (PCL) was used to fabricate polymerised high internal phase emulsions (polyHIPEs)² scaffolds to support 3D breast cancer (MDA-MB-231) cell culture. The effect of varying properties, such as mixing speed and the inclusion of gelatin in the internal phase, on polyHIPE porosity, interconnectivity, morphology, and mechanical properties was assessed via SEM and tensile testing. MDA-MB-231 cells were used to assess cell morphology, metabolic activity, and migration on 4PCLMA polyHIPE scaffolds. An ex ovo chick chorioallantoic membrane (CAM) assay was used to assess the biocompatibility of the polyHIPEs and potential for vascular invasion.

RESULTS: Via varying mixing speed and the content of gelatin within the internal phase the creation of interconnected micro and macro pores with varying mechanical properties was achieved, successfully demonstrating the tunability of these polyHIPEs. Ex ovo CAM assays identified the scaffolds as bioinert, with biocompatible properties within a vascularised tissue. Furthermore, in vitro assessment of cell viability and proliferation showed promising potential for the use of PCL polyHIPEs to support breast cancer cell growth and attachment.

DISCUSSION & CONCLUSIONS: Photocurable high molecular weight 4PCLMA polyHIPE scaffolds show promise as a substrate for breast cancer cell culture with potential for vascularisation and active perfusion due to ease of tunability.

ACKNOWLEDGEMENTS: The EPSRC, centre for doctoral training in Advanced biomedical Materials for PhD studentship funding (EP/S022201/1)

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Keywords: Polymers - natural / synthetic / responsive,

PP-017

Collagen-nanoclay hydrogels for stiffness enhancement of the extracellular matrix *in vitro*

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INTRODUCTION: Physiologically, cells are embedded within a fibrous extracellular matrix (ECM); whose main protein is collagen. This matrix mechanical properties not only allow the tissues to withstand stresses, but also regulate numerous cellular functions. In addition, increased tissue stiffness is associated with pathologies, including cancer and cardiovascular disease. ECM stiffness is emerging as a mechanical cue that precedes disease by altering cellular behaviors¹. Consequently, being able to tailor ECM stiffness could be a powerful method for personalized research and therapies in tissue engineering.

Clay nanomaterials are an emerging biomaterials of interest due to their atomically thin layered structure, charged characteristics, biocompatibility and composition². Here, we hypothesized that nanoclay, once they are attached to a collagen-based hydrogel, may provide a superior stiffness without significant micro-structural changes in the hydrogel architecture. Therefore, laponite nanoclays (LapNC) were added to collagen hydrogel of different concentrations and its mechanical properties were measured to demonstrate the stiffness increase and to perform a thorough characterization. In addition, we propose these hydrogels of collagen and LapNC as 3D cancer culture systems for stiffness-dependent tumor spheroid growth.

METHODS: Collagen hydrogel at 2.5, 4 and 6 mg/ml (collagen type I rat tail) were supplemented with LapNC solution in culture medium. The hydrogels were observed under scanning electron microscopy (SEM) to qualitatively see their microarchitecture, their storage moduli were measured by rheology, and other physical parameters as permeability and swelling ratio were quantified. Cell viability was also evaluated and tumoral cells were cultured in LapNC supplemented and control hydrogels to track multicellular structures formation.

RESULTS: Laponite solutions were achieved in the culture media without clay aggregation and the supplemented hydrogel did not show any collagen collapse owing to the presence of the clays. The characterization study confirmed the enhancement of the mechanical properties of the supplemented hydrogels, without compromising cellular viability. Regardless of the collagen concentration, LapNC supplemented hydrogels exhibited higher storage moduli and lower permeability. The addition of LapNC to the hydrogel involved the formation of larger spheroids.

DISCUSSION & CONCLUSIONS: LapNC addition enhanced mechanical properties of the collagen matrix. These results widens the usage of these hydrogels for new tissue engineering applications, such as cancer spheroids analysis.

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Keywords: Biomaterials, Microenvironment and niche engineering

PP-018

Development of electroactive composites for the electrical stimulation of stem cells

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INTRODUCTION: There is a need for reliable whole organ models of the spinal cord. The design of such models requires the use of appropriate (stem) cells, polymeric materials and biophysical stimuli. Electrical stimulation (ES) is a promising biophysical stimuli for tissue engineering strategies. The phenotype of the stimulated cells (e.g., neurons, astrocytes, osteoblasts) resembles that of the living tissue.

METHODS: Through the combination of different biofabrication techniques (e.g. electrospinning, 3D-printing, hydrogel), it is possible to create artificial tissue microenvironments capable of supporting different cells. These can later assemble into whole organ models.

RESULTS: Our group has worked on different material-based approaches to create suitable scaffolds to perform ES of stem cells. These can be merged together to create a complex tissue model of the spinal cord and vertebrae. Some of the strategies include:

- (a) the production of poly(3,4-ethylenedioxythiophene) (PEDOT)-based biodegradable/biodispersible composites adequate for 3D-printing and/or electrospinning;
- (b) doping of polybenzimidazole (PBI) nanofibers;
- (c) cross-linked poly(acrylonitrile) (PAN)-PEDOT fibers;
- (d) poly(ε-caprolactone) (PCL) – poly(aniline) (PANI) / poly(glycerol sebacate) (PGS) coaxial fibers;
- (e) Conductive and bioactive hydrogels.

DISCUSSION & CONCLUSIONS: We found that electroconductive polymers are paramount for designing a biomimetic artificial spinal cord model capable of ES. Both neural and bone cells derived from iPSCs and MSCs differentiate better with ES. Our results support the creation of artificial models of the spinal cord.

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Keywords: Additive manufacturing, Electroactive materials

PP-019

Modulation of the pseudo-doping effect in polyaniline-based electrospun fibers

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INTRODUCTION: Electroconductive polymers (ECPs) are critical for the design of soft electronic and bioelectronic devices. This arises from a combined electronic/ionic conductivity, which lowers the impedance of devices, easier processability when compared with metals and low-cost/ease of production. Poly(aniline):Camphorsulfonic acid (PANI:CSA) is an example of a biocompatible ECP, whose electroconductivity is highly dependent on chain organization. As such, performance of PANI:CSA based soft electronic devices directly depends on the processing method used.

Trifluoroethanol (TFE) and hexafluoropropanol (HFP) are two solvents capable of improving PANI:CSA chain organization (pseudo-doping effect), but no systematic studies were conducted to assess their performance. The goal of this work was to study and develop a solvent system capable of improving the electrical properties of PANI:CSA-based materials for soft electronic applications.

METHODS: PANI:CSA pellets and spin-coated films were used to evaluate electroconductivity and respective structural changes associated to pseudo-doping. Different solvents were studied, including m-Cresol (positive control), chloroform (negative control), TFE, HFP and different TFE/HFP mixtures. Electroconductivity was determined using a 4-probe method, whereas PANI:CSA chain conformation was evaluated through a combination of SEM, AFM, Raman Spectroscopy, UV/Vis/NIR, FTIR, XPS and XRD.

PANI:CSA was also incorporated into electrospun poly(caprolactone) to create electroconductive scaffolds for neural tissue engineering. TFE and mixtures of TFE and HFP were tested. Fibers were characterized for fiber diameter, electroconductivity mechanical properties, contact angle and biocompatibility. The best sample was used to study the effect of ES on the proliferation and differentiation of cultured neural stem cells (ReNVM Cells).

RESULTS: Samples obtained by spin-coating showed greater improvements in electroconductivity (TFE = 61 S cm⁻¹, TH55 = 70 S cm⁻¹), attributed to faster solvent removal. Raman and AFM evidenced different chemical domains with different chain organizations, XPS evidenced differences in CSA doping efficiency, UV/Vis/NIR indicates the formation of conduction bands with different efficiencies.

DISCUSSION & CONCLUSIONS: PCL-PANI:CSA fibers were electrospun using the strategy envisaged. When TFE alone was used, electroconductivity peaked 0.042 S cm⁻¹ before reaching a plateau. Electroconductivity improved when HFP was added and peaked when the solvent system TH55, containing 50%(v/v) TFE and 50%(v/v) of HFP, was used (0.2 S cm⁻¹). Differentiating NSCs cultured

under ES on TH55 sample aligned with the direction of the electrical field and had increased expression of neural markers DCX, MAP2 and S100 β .

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REFERENCES:

Keywords: Polymers - natural / synthetic / responsive, Interfaces - engineered

PP-020

Bone Clip: Developing an angiogenic biomaterial to increase bone healing

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INTRODUCTION: The management of delayed bone healing injuries, namely critical size defects and non-union fractures, represents a substantial clinical burden. Treatments for other bone related injuries such as autografts and allografts provide poor long-term outcomes when applied to these injuries, with high rates of complications and reoperations, and low functionality reported¹. The present work studies how the periosteum could be used to address this clinical challenge. The periosteum is the thin, fibrous membrane that envelopes non-articular bone and plays a significant role in the natural bone healing process. It is highly vascularised and bi-layered, with the innermost layer acting as a mesenchymal stem cell niche. Despite its known importance, the periosteum remains relatively unexplored within literature. In this project, we aim to develop and evaluate a periosteal bone substitute to tackle delayed bone healing. The so called 'bone clip' will be made from synthetic and easy to handle polymers and will be loaded with proangiogenic factors to stimulate angiogenesis and accelerate bone regeneration by mimicking the function of the periosteum. It is our intention that the bone clip will be used in conjunction with existing surgical procedures and in addition to void fillers to stimulate the natural healing process.

METHODS: To date, we have investigated the manufacture of scaffolds with multiscale porosity to promote angiogenesis driven bone formation using polycaprolactone (PCL) and poly(glycerol sebacate) (PGS) polymerised high internal phase emulsions (polyHIPEs). The periosteal substitute material needs to be highly porous to encourage endogenous cell migration and to be easy to load with proangiogenic agents if required. These polyHIPE scaffolds were manufactured via emulsion templating and mesenchymal stem cells were cultured onto them to assess cytotoxicity, attachment, viability, and proliferation.

RESULTS: The cells successfully attached and proliferated over the course of seven days, and confocal microscopy revealed the migration of cells into the porous structure over time, demonstrating the angiogenic potential of the scaffolds.

DISCUSSION & CONCLUSIONS: Overall this study has so far demonstrated that PCL and PGS polyHIPEs provide a promising approach to producing an angiogenic periosteum substitute for delayed healing bone injuries. The next step of this project will be to tailor the porous structure of the scaffolds to include micrometre-scale porosity using additive manufacture techniques to further enhance vascular ingrowth, and to load the scaffold with proangiogenic factors such as 2-deoxy-D-ribose (2dDR).

ACKNOWLEDGEMENTS:

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

PP-021

Screening Poly(Beta-Amino Ester) Nanoparticles for mRNA Delivery to Primary Human Skin Cells for Lymphangiogenesis

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INTRODUCTION: The lymphatic system controls fluid homeostasis, drainage, lipid absorption, and immune cell transport. When the lymphatics are impaired it can result in secondary lymphoedema, which affects 15% of cancer survivors¹. Promoting lymphangiogenesis using modified mRNA encoding for Vascular Endothelial Growth Factor C (modVEGF-C) is a promising approach to restore the lymphatic network from pre-existing lymphatic vessels, compared to the limited effect of recombinant VEGF-C delivery due to its short half-life².

However, non-viral delivery vectors display low cell specificity and limited biodistribution, accumulating mainly in the liver or spleen³. In addition, primary cell transfection continues to pose a challenge⁴, with some cell types remaining understudied. Poly(β -Amino Ester)(PBAEs) are biodegradable, cationic polymers that can be synthesised from chemically diverse acrylate and amine monomers. Focusing on the target of lymphatic vessels present in the dermis, we aimed to identify a formulation for effective transfection of human skin cells with reporter mRNA.

METHODS: A library of 72 PBAEs were synthesised via Michael addition combining 4 diacrylates, 3 backbone diamines and 6 end-cap amines. PBAEs were complexed with Firefly luciferase(FLuc) mRNA and transfection efficiency, cytotoxicity, and cell viability assessed in primary human skin cells *in vitro* 24 hours post-transfection. Promising candidates were further formulated with polyethylene glycol (PEG)⁵ to evaluate its influence on particle stability using dynamic light scattering.

RESULTS: Herein, we report a set of PBAE vectors which successfully delivered FLuc mRNA to two different primary human skin cell types *in vitro* comparable to a commercial control. Interestingly, altering PBAE chemistry influenced transfection in a cell-specific manner. Viability and cytotoxicity assays showed metabolically active cells 24 hours post transfection. A lead PBAE candidate was selected to study the effect of further PEGylation. Surface zeta potential and hydrodynamic size measurements showed a decrease in the polyplex charge and an enhanced stability over time at room temperature.

DISCUSSION & CONCLUSIONS: Overall, this work indicates that PBAE vectors can deliver mRNA to hard-to-transfect primary cells from human skin. Future studies will optimise the formulation to enable sustained release of modVEGF-C to promote lymphangiogenesis.

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Keywords: Polymers - natural / synthetic / responsive, Nanomaterials (inc graphene)

PP-022

Development and characterisation of new bioelastomers based on poly(glycerol sebacate)-co-polyethylene glycol for biomedical applications

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INTRODUCTION: Poly(glycerol sebacate) (PGS), a synthetic biorubber, is characterised by its biocompatibility, high elasticity and tunable mechanical properties; however, its inherent hydrophobicity and insolubility in water make it unsuitable for use in advanced biomaterials such as hydrogel fabrication. Here, we have developed new hydrophilic PGS-based copolymers that enable hydrogel formation. Poly(glycerol sebacate)-co-polyethylene glycol (PGS-co-PEG) copolymers were developed by two different types of polyethylene glycol (PEG), combined at different ratios. In addition, functionalised PGS-co-PEG was prepared with methacrylate groups, leading to photocurable copolymers that can quickly crosslink to form an insoluble matrix. By combining hydrophilic PEG with hydrophobic PGS, we have demonstrated that it is possible to create hydrogels with outstanding bioadhesiveness, elastomeric properties, and pH-responsive behaviours.

METHODS: Polyethylene glycol 1000 g/mol (PEG2) and glycerol ethoxylate 1000 g/mol (PEG3) as two different types of PEG were used. A two-step polycondensation reaction was then used to produce PGS-co-PEG copolymers. Further, the methacrylation was carried out to develop photocurable copolymers. To characterise these copolymers, two crosslinking methods were used: thermal crosslinking for non-functionalised PGS-co-PEG and photo-crosslinking for functionalised PGS-co-PEG (M(PGS-co-PEG)).

RESULTS: This results in PGS-co-PEG and M(PGS-co-PEG) amphiphilic polymers. We have shown that the properties of these copolymers can be controlled by altering the type and amount of PEG and crosslinking techniques. For example, both PGS-co-PEG and M(PGS-co-PEG) copolymers containing PEG ≥ 40% showed high swelling, flexibility, stretching, bioadhesion and biocompatibility, and good enzymatic degradation and mechanical properties. Also, the addition of PEG created hydrogels with pH-responsive behaviours. These hydrogels can be used for bioapplications requiring responses to physicochemical dynamics. Interestingly, samples including 40% and 60% PEG had the highest lap-shear strengths, although thermally crosslinked PGS-co-PEG had higher shear strengths than photo-crosslinked M(PGS-co-PEG) specimens. The obtained shear strengths are comparable to those of commercially available sealants/bioglues.

DISCUSSION & CONCLUSIONS: Due to the versatile multifunctionalities of these new copolymer hydrogels, they have great potential in soft tissue engineering and biomedical applications. Also, as a result of the development of these multifunctional copolymers, smart biomaterials for biomedicine may be developed in the near future.

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Keywords: Biomaterials, Bioadhesives

PP-023

Methacrylate silk fibroin sponges as candidates for bone tissues substitutes

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INTRODUCTION: Silk fibroin, extracted from silkworm cocoons, exhibits fascinating properties as biodegradability, low cost, ease of fabrication, tunable mechanical properties, and degradation kinetics, among others. It is widely used in tissue engineering and regenerative medicine fields (1). Thus far, one of silk fibroin's extraordinary properties is its capability to promote MSCs differentiation toward osteogenic phenotype, making it a good candidate for bone tissue regeneration applications, both in vitro and vivo². Different silk fibroin-based scaffolds have been investigated overtime, as porous sponges prepared via salt leaching, freeze-drying or gas-foaming, methacrylate silk fibroin sponges, printed scaffold, hydrogels (3,4). Silk fibroin's outstanding and versatile properties allow an easy control of the pore dimension, degradation rates, and mechanical properties while providing proper biological environment for cell differentiation, thus being a good candidate for bone applications⁵. Among the different crosslinking processes, photo-crosslinking allows the formation of covalent, stable bonds in a controlled and precise manner¹.

In this work, photo-crosslinked methacrylate silk fibroin sponges have been evaluated as bone substitute candidates. Specifically, the sponges were fabricated with different photo-initiator concentrations and in presence/absence of a porogen to investigate the impact of the composition on the pore size, mechanical properties, and stem cells differentiation.

METHODS: Biological evaluation was performed by testing human mesenchymal stem cells bone marrow-derived (hBM-MSCs) differentiation toward osteogenic phenotype over 21 days culture time. Osteogenic differentiation was studied by measuring over time alkaline phosphatase activity (ALP), collagen I deposition by imaging, as well as osteogenic differentiative markers expression through PCR. Moreover, the impact of sponges' compositions on pore dimension has been investigated via electron microscopy while mechanical compressive properties and silk secondary structure rearrangement via infrared spectroscopy were also studied.

RESULTS: The study demonstrated that the different sponges' compositions, thus the presence/absence of porogen and different photo-initiator concentrations did not affect silk fibroin secondary structure. Moreover, the lowest photo-initiator concentration in combination with the porogen led to widest pore distribution and enhanced osteogenic differentiation as confirmed by gene expression tests.

DISCUSSION & CONCLUSIONS: All the compositions supported cell differentiation toward osteogenic phenotype and the pore size can be controlled by tuning sponges' composition. A significant positive effect on pore dimension and osteogenic differentiation was obtained in presence of the porogen which might be due to stabilization of air bubbles inside silk fibroin sponges.

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(3) <https://doi.org/https://doi.org/10.3390/ma14030674>

(4) <https://doi.org/https://doi.org/10.3390/molecules27092757>

(5) https://doi.org/https://doi.org/10.1007/978-981-13-0947-2_20

Keywords: Bone and bone disorders (osteoporosis etc), Differentiation

PP-024

Physical gelation as an additional tool to modulate low viscosity GelMA for hydrogels structuring

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INTRODUCTION: Gelatin methacryloyl (GelMA) hydrogels potential for tissue engineering has been well established, showing high biocompatibility and integration in vivo. However, there is still a lack of information in the understanding of GelMA molecular configuration and how to use this information to tailor hydrogels with defined properties, information that is very relevant for the design of functional hydrogels. It has been described that methacryloyl functionalization produces a decrease in viscosity, gelation and melting temperatures of the original gelatin used for functionalization, however, this temperature dependency and their effect on the structural and mechanical properties, as well as the stability of the resultant hydrogels have not been completely explored. Due to this, the objective of this work is to determine the effect of physical crosslinking on the mechanical properties of GelMA hydrogels.

METHODS: We produced GelMA with different degrees of substitution (DS) as well as using gelatin from two different origins (porcine and salmon), that present distinctive sol-gel temperatures, to obtain 6 different GelMA materials for further molecular, rheological, and thermal characterization. GelMA hydrogels were produced using 7% GelMA suspensions (pH 7.4) with 0.3% IRGACURE 2959. Before crosslinking, suspensions were preincubated at 4, 20 or 37°C for 1h and then photo-crosslinked by exposing them to UV light (365 nm). Hydrogels were characterized by determining their mechanical and thermal properties.

RESULTS: It was possible to determine changes in molecular vibrations in gelatin due to functionalization using RAMAN spectroscopy. GelMA rheological analysis showed differences between the different samples. Higher G' values and viscosity were obtained in porcine GelMA (PGelMA) in comparison with salmon GelMA (SGelMA) samples at temperatures lower than 20°C. Also, gelation temperatures were reduced when increasing the DS in gelatins from both origins. DSC data complemented this information, showing a decrease in gelation and melting enthalpy as DS increased, as well as differences between GelMAs from different origins. Mechanical characterization of GelMA hydrogels evidenced that the temperature dependency observed for GelMA samples during rheology condition the mechanical properties of the generated hydrogels, especially in the case of PGelMA hydrogels, showing an increase in compression moduli when physical crosslinks are promoted prior to photo-crosslinking. In addition, DSC measurements suggest that these hydrogels are thermally stable, independent of the level of physical crosslinking that is promoted.

DISCUSSION & CONCLUSIONS: We were able to determine the contribution of physical crosslinking on GelMA hydrogels mechanical properties, suggesting that it is possible to modulate these hydrogels structure by only previously tuning physical gelation.

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REFERENCES:

Keywords: Biomaterials, Hydrogels and injectable systems

PP-025

Osteoblast behaviour on keratin modified titanium surface

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INTRODUCTION: Surface modification of titanium by coating with natural bioactive polymers, has been suggested as a means to improve osseointegration in dental implantology applications. Wool-derived keratin, due to its demonstrated ability to promote bone formation, has been suggested as such a potential bioactive material. As limited knowledge is available on the biological effects of keratin on osteoblasts, the aim of this study was to assess the effects of two novel methodologies used to coat titanium surfaces with keratin, on osteoblast function.

METHODS: Titanium was silanized with APTES and crosslinked with glutaraldehyde to facilitate covalent binding with keratin. Two keratin-coated titanium surfaces were fabricated via solvent casting (Ti-KC) and functionalization (Ti-KF) respectively. The attachment, proliferation, viability and cellular morphology of MG-63 osteoblast cells cultured on these modified surfaces were determined by semi-automated quantitative microscopy. The osteogenic effect of the modified surfaces on osteocalcin (OCN), osteopontin (OPN), alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2), Collagen Type I (COL1) and bone morphogenetic protein-2 (BMP2) gene expression was investigated by quantitative PCR.

RESULTS: MG-63 proliferation over 14 days on the Ti-KF surface was the same as on uncoated titanium. On the Ti-KC surface, proliferation initially lower at days 1 and 7 was similar to control levels by day 14. No significant differences in cell viability were seen. Elongated spindle-shaped cells were seen on functionalized and untreated titanium on day 1, whereas oval-shaped cells were noted on the coated surface. Over time in culture however, MG-63 cells on all surfaces became spindle shaped with no statistical difference in morphological measurements. Osteogenic gene expression on the modified surfaces was higher compared to uncoated titanium at all times. While a similar temporal gene expression over the 14 days was noted on both surfaces, osteocalcin (OCN) expression on the Ti-KC surface was significantly increased over the first three days compared with that on the Ti-KF surface. OPN, ALP, RUNX2, COL1 and BMP2 gene expression was similar on either coating.

DISCUSSION & CONCLUSIONS: The surface topography and chemistry of biomaterials is known to influence a wide range of biological responses such as cell migration, adhesion, proliferation, and differentiation in adherent cells. This study showed that covalently bound keratin on titanium surfaces did not have any lasting negative impacts on MG-63 cell attachment, morphology or subsequent proliferation. Improved osteogenic gene expression in MG-63 cells as a result of keratin coating suggests this surface may be useful in bone-implant applications.

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REFERENCES:

Keywords: Biomaterials, Imaging - advanced

PP-026

Evaluation of Fibrin/PolyVinyl Alcohol Scaffolds for Skin Tissue Engineering by Emulsion Templating

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INTRODUCTION: Tissue scaffolds which support regenerative reconstruction of skin and other connective tissues must provide a combination of physical biochemical and biological properties. The balance of properties in a scaffold product represents an empirically or theoretically based design strategy.

To address the need for rapid integration in skin reconstruction, we initially identified fibrin empirically, as a pro-vascular substratum. In order to create a highly porous three-dimensional architecture, we developed an emulsion templating methodology compatible with native protein structure and activity.

Here, we hypothesised that the highly hydrophilic polymer polyvinylalcohol (PVA), may confer supportive bulking properties to a fibrin-based scaffold as a biocompatible physical conjugate.

METHODS: Fibrin and Fibrin/PVA scaffolds were formed by thrombin coagulation of fibrinogen, in the presence of PVA, and an emulsion phase as the porogen, with glutaraldehyde as the cross-linking agent. After freeze drying, the scaffolds were characterized and evaluated for biocompatibility and efficacy of dermal reconstruction in a murine skin excision model.

RESULTS: SEM analysis showed all scaffolds had an interconnected porous structure (pore size up to 330 μm), and preserved nano-scale fibrous architecture of the fibrin, with PVA forming an interpenetrating reticular structure. FTIR analysis revealed new H-bonds present in fibrin/PVA composites, suggesting non-covalent composite formation. The mechanical properties of the scaffolds were investigated by tensiometric testing, showed the scaffolds' ultimate tensile strength around 0.12 MPa with an elongation around 50%. The proteolytic degradation of the scaffolds was quantified by mass decrease over time, and found to be controlled over a wide range by varying the type or degree of cross-linking, and by fibrin/PVA composition. Scaffold cytocompatibility was assessed by proliferation of human mesenchymal stem cell (MSC) proliferation. Results show that MSC attachment, penetration and proliferation in scaffolds is enhanced by PVA at an optimal ratio. The efficacy of scaffolds for tissue reconstruction were evaluated in a murine full-thickness skin excision defect model. The scaffolds were integrated and resorbed without inflammatory infiltration, and compared to control wounds, promoted deeper neodermal formation, greater collagen fibre deposition, facilitated angiogenesis, and significantly accelerated wound healing and epithelial closure.

DISCUSSION & CONCLUSIONS: The experimental data showed that PVA in a fibrin composites may support vascularisation, increase proteolytic stability, and regenerative outcome.

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REFERENCES:

Keywords: Biomaterials, Wound healing

PP-027

Enhancing Biocompatibility, Antibacterial Characteristics and Corrosion Rate of Pure Zinc by Surface Acid Etching

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INTRODUCTION: Bioresorbable metals are promising candidates in the field of regenerative medicine for promoting tissue repair without the adverse effects from a lifelong presence. Zinc (Zn) is a biodegradable metal with great potential as a medical device material, which has gained particular interest due to its tuneable mechanical properties and desirable biocompatibility [1]. However, the slow degradation rate of Zn under physiological conditions does not meet the ideally requirements for biodegradable implants, hindering its clinical translation. The present study demonstrates a novel and simple surface acid-etching modification technique to accelerate the corrosion rate of pure Zn induced by the roughened surface and increased surface area. Our approach represents a viable and effective strategy to impart pure Zn with accelerated corrosion behaviour, increasing the translation potential of zinc for various biomedical applications.

The project aimed to:

1. Accelerate the corrosion rate of Zn through acid etched increased surface roughness.
2. Assess the mechanical, chemical, and physical properties of the etched surface.
3. Understand the cellular and bacterial response to untreated and acid-etched Zn.

METHODS: High-purity Zn rolled sheets were wire-cut and mechanically wet-ground. The samples were immersed at 37 °C and 100 rpm in a 1:1:1 solution of sulfuric acid: hydrochloric acid: water, (H₂SO₄/HCl/H₂O) for 1, 3, and 5 min. The effect of acid etching on surface morphology, surface roughness, mechanical properties, electrochemical and degradation behaviour, cytocompatibility, direct cell attachment, and biofilm formation was investigated.

RESULTS: Acid-treated Zn showed an exceptionally high rate of corrosion (~226-125 µm/year) compared to untreated Zn (~62 µm/year), attributed to the increased surface roughness (Ra ~ 1.12 µm). Immersion tests in Hank's solution revealed that acid etching accelerated the degradation rate of Zn samples. In vitro, conditioned media supplemented with 25% and 50% of media extract from etched samples demonstrated good cytocompatibility with the murine osteoblast cell line MC3T3-E1. Reduced bacterial adhesion, biofilm formation, and dispersion was observed for *Staphylococcus aureus* biofilms cultured on acid-etched pure Zn substrates.

DISCUSSION & CONCLUSIONS: Collectively, our novel findings support the feasibility of acid-etched pure Zn substrates in the physiological environment and suggest that these surface-treated materials are suitable for orthopedic implants and devices.

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Keywords: Interfaces - engineered, Bone and bone disorders (osteoporosis etc)

PP-028

Bioadhesive Microcapsules as a New Cellular Treatment for the Diffuse Cartilage Lesions

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INTRODUCTION: Diffuse cartilage lesions affect a broad area of the articular cartilage and have a high prevalence as the common type of degenerative processes. Diffuse damage is usually restricted to palliative care or systemic anti-inflammatory treatments up to the point where the majority of the cartilage is lost, when the only option available is the surgical replacement of the arthritic joint with a prosthesis.

Cell-based approaches using autologous chondrocytes or mesenchymal stem cells (MSCs) [1] have been tested and it can even be found in some clinics. However, the efficacy of such cell-based treatment is controversial [2] where the main problem is the unconfinement of the cells used. In general terms, the applied cells stay in the injection site only for short periods of time, so their regenerative potential is greatly reduced. For this reason, the most promising approach is the combination of such cells with a biomaterial-based carrier that can play that role.

In order to resolve the previous task, the general objective of this study is the development of bioadhesive and injectable cell microcarriers with the ability to regenerate the articular cartilage.

METHODS: Liquid overlay technique was used to obtain MSCs spheroids, this method inhibits the attachment of cells to tissue culture plates and promotes cell-cell aggregation. Microcapsules are based on novel advanced and multibiofunctional protein-based polymers, obtained by recombinant DNA technology and named Recombinamers, more specifically a type of them, the Elastin-like Recombinamers (ELRs), designed for this application [3]. In addition, advanced crosslinking chemistry such as 'catalyst-free click chemistry' is integrated in MSCs-spheroid encapsulation to produce a reactive layer-by-layer technology.

RESULTS: The aim of this study is materialized in the fabrication of multibiofunctional microcapsules that are able to promote the cell cargo with selective adhesion and location on the articular surface. To do so, ELRs were designed and bioproduced to allow cell-material interaction on both the inner and outer surface of the microcapsule by means of the inclusion of, on one hand, specific cell adhesion domains and, on the other hand, sequences that promote the adhesiveness to collagen II and chondroitin sulfate. Besides, 3 ELR-bilayers were enough to coat MSCs spheroids.

DISCUSSION & CONCLUSIONS: Such coating allows to achieve a nanometer-thin but strong and highly efficient conformational coat on the cell spheroids so a new cell-based therapeutical approach can be developed.

ACKNOWLEDGEMENTS:

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Keywords: Cartilage / joint and arthritic conditions,

PP-029

Functionalisation of modified Ti-6Al-4V surfaces with antimicrobial peptide KR12 to create antibacterial surfaces

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INTRODUCTION: The unwanted microbial colonization of implant surfaces following surgical orthopaedic procedures can result in infection, preventing implant integration. Antibiotics have limited activity, more so if biofilm or an antibiotic-resistant microorganism is present¹. Coatings using immobilized molecules can improve the antimicrobial properties of implants. Antimicrobial peptides (AMPs) are promising candidates, because of their broad-spectrum antimicrobial activity, low toxicity, and less likelihood to induce resistance². In this work, using titanium alloy Ti-6Al-4V (Ti64) as a model material, a small peptide sequence with electrostatic affinity for Ti64 will be added to an AMP sequence to create a solid-binding antimicrobial peptide with affinity to implant surfaces³. Metal surfaces will also be submitted to a combination of mild chemical and heat treatments and laser patterning to favour electrostatic attachment by increasing oxide species on the surface and producing surface morphology more suitable for bioactivity.

METHODS: First, Ti-6Al-4V samples 10x10x1.6 mm³, were polished to mirror-like finish. Then, to electrostatically immobilize the modified AMP KR-12 (smallest fragment of human cathelicidin LL-37), samples were submitted to a combination of: chemical treatment – CT, by immersion in 10 mL of H₂O₂ 8.8 M, HCl 0.1 M at 80 °C for 30 minutes; heat treatment – HT, by thermal oxidation at 450, 550, 650 and 750 °C for 60 minutes; laser patterning – LP, using different parameters of laser power (30, 60 and 95 mW) and speed (0.125, 0.25 and 0.5 mm s⁻¹).

RESULTS: Physicochemical characterization of modified surfaces using Raman spectroscopy, XRD, AFM, WCA, SEM, EDX were performed. Combined CT and HT at higher temperatures increased the surface oxide layer (increased contributions of TiO₂ crystalline phases and percentage of surface oxygen), induced higher roughness values and lower WCA when compared to polished surfaces and treatments at lower temperatures. Laser patterning did not alter considerably the oxide layer and WCA, but some parameter combinations increased the percentage of surface oxygen; surface roughness values were dependent on specific combinations.

DISCUSSION & CONCLUSIONS: New methods to prevent pathogenic bacteria via self-disinfecting surfaces coated with AMPs can provide alternatives to the use of antibiotics. The purpose of this study is to produce new and simple functionalization procedures to prevent microbial colonization and consequence complications.

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Keywords: Composite materials, Infection

PP-030

Sustainable Polyhydroxyalkanoates-based 3D printed scaffolds with antibacterial properties for bone tissue engineering

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INTRODUCTION: Bone tumour removal, traumas with large defects, and degenerative diseases are the main catastrophic events impeding complete bone healing [1]. To overcome current treatments limitations [2], we created 3D printed composite scaffolds based on a combination of Poly(3-hydroxybutyrate) and a Zinc-doped Borosilicate-based bioactive glass (BS-Zn) to support bone tissue regeneration and provide antibacterial activity. [3]

METHODS: P(3HB) was produced by bacterial fermentation of *B. sacchari*. Solvent casting has been used to produce composite films with different bioactive glass concentration (v/v%) to select the best composition to be used for the development of 3D printing scaffolds with different geometry using Fused Deposition Modelling.

MG63 human osteoblast cell line was cultured on the composite and neat polymer films to assess biocompatibility and proliferative activity through Resazurin and Live/Dead assays. Primary Osteoblasts were used to investigate the mineralisation activity induced by the scaffolds. To evaluate the antimicrobial activity, MIC and MBC were determined against *E. coli*, and *S. aureus* for P(3HB)/BS-Zn scaffolds.

RESULTS: Chemical and thermal analysis confirmed that the polymer produced was Poly(3-hydroxybutyrate) with very similar properties to previous studies and commercially available products [4]. The addition of bioglasses induced a nanostructured topography on the surface of the composites and the production of hydroxyapatite, as visible by SEM. The resazurin assay demonstrated the biocompatibility of P(3HB) and the related composites. 3D printed composite scaffolds were successfully developed with high reproducibility, showing high level of mineralisation towards primary osteoblasts cells.

The MIC/MBC test confirmed the antimicrobial activity of the composites.

DISCUSSION & CONCLUSIONS: In this study, 3D printed composite scaffolds using a short-chain length PHA with high v/v% of Zn-doped bioglass have been successfully synthesised. The biocompatibility of the neat materials and the composite scaffolds have been confirmed, and the mineralisation activity investigated towards primary osteoblasts cells. Moreover, the antimicrobial activity of P(3HB)/BS-Zn scaffolds were investigated. The final aim of this project is the development of a promising composite material with suitable physicochemical properties and antimicrobial activity for bone tissue engineering applications.

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Keywords: Biomaterials, Composite materials

PP-031

Dual Action Antimicrobial Bone Regeneration Paste

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INTRODUCTION: There is a pressing unmet clinical need for biomaterials that are able to promote bone regeneration whilst preventing bone infections. Nanoscale hydroxyapatite (nHA) pastes are available on the market with promising injectable and osteostimulative properties, but without antimicrobial properties. To introduce this clinically important feature, a novel “dual action” bone regeneration paste has been designed, introducing strontium into the hydroxyapatite crystal lattice for further stimulation of bone regeneration and zinc oxide for antibacterial properties [1].

METHODS: The dual action paste (DAP) was prepared using in-house methods (Ceramisis Ltd., UK) and a nHA paste equivalent to clinically available ReproBone® novo was used as a reference. Characterisation techniques including x-ray diffraction and transmission electron microscopy were performed. A biofilm initialisation method was developed to quantify the number of viable bacteria (*S. aureus* clinical isolate) attached to the pastes after 24h, thereby assessing the antibacterial activity of the pastes. In vitro biocompatibility was tested with pastes present in inserts above a monolayer of MG63 human osteosarcoma cells. In vivo biocompatibility and bone regeneration potential were examined by implanting the pastes for 6, 12 and 19 weeks in a rabbit femoral defect model.

RESULTS: The DAP was composed of phase pure strontium-substituted hydroxyapatite and zinc oxide. The biofilm initialisation model showed reduction in viable bacterial numbers of over 3-log for the DAP. Promising in vitro biocompatibility results were obtained with no significant reduction in viability detected between the cells exposed to DAP and the control cells. Analysis of the rabbit femoral condyles after in vivo implantation showed that DAP was able to beneficially maintain the bone void volume, preventing soft tissue ingrowth. Histological analysis showed the formation of new bone directly on the surface of the DAP. No systemic adverse effects were observed in the brain, liver, lungs and kidneys after 19 weeks of implantation.

DISCUSSION & CONCLUSIONS: DAP showed great potential to reduce the incidence of bone infections whilst promoting bone tissue regeneration. Therefore, this new biomaterial shows promise to meet the pressing unmet clinical need urgently required to improve patients’ quality of life alongside reducing pressures on healthcare systems. Future work will focus on the testing required to proceed to clinical trials as a new medical device.

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REFERENCES: 1. WO2020044028A1 patent application, priority date 28/08/18.

Keywords: Advanced therapy medicinal products, Bone and bone disorders (osteoporosis etc)

PP-032

Effect of HA Particle Size and Quantity on The Bioactive and Biological Behaviour of PU Scaffolds

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INTRODUCTION: In maxillofacial area, specifically orbital floor, injuries can cause bone deformities that are difficult to repair or regenerate. Treatment methodologies include use of polymers, metal, ceramics on their own and in combinations, but more attention has been paid to identify suitable materials for orbit floor regeneration.

METHODS: The bioactive PU/HA scaffolds, either micro or nanoparticles size, were prepared via the articles leaching technique. Different percentages of HA (25%, 40% and 60%) were used to evaluate the most suitable percentage for orbital floor repair and regeneration. The mechanical, physical, chemical and biological characterisation of scaffolds were investigated by tensile tests, SEM, FTIR, Cell viability (using MG-63), and CAM assay.

RESULTS: FTIR-PAS characterization confirmed the presence of HA in composite scaffolds, while ATR confirmed the significant amount of HA at the scaffolds top surface in micro-HA. However, nano-HA had a better disparity than micro-HA. The SEM images confirmed the interconnectivity. Increasing the content of HA up to 40% led to improve the mechanical properties, but nano-HA was more promising than that of micro-HA. The 60% HA made the scaffolds so hard and easy to be broken. The cell viability (using MG63) showed no significant difference between PU and PU/HA scaffold either micro or nano. For CAM assay, there were significant different between PU and PU/HA scaffolds, where PU/HA scaffolds showed more vascularisation.

DISCUSSION & CONCLUSIONS: This study describes the PU/HA bioactive scaffold neovascularization potential. The development of new biocompatible materials with tailored properties will be useful in tissue engineering especially in orbital floor regeneration. HA nano particles were equally disrupted on the PU scaffold surfaces compared to HA micro. Leading to better mechanical strength. Besides, cell viability was higher with the increase of HA percentage. In addition, the PU/HA nano 40% scaffolds might be the most suitable for orbital floor, due to mainly the mechanical properties, as well as the results of cell viability, new collagen formation and enhanced vascularisation.

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Keywords: Biomaterials, Composite materials

PP-033

Electrical stimulation: Use of a high throughput electrical stimulus bioreactor to study the best regimes for *in vitro* cellular mechanisms

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INTRODUCTION: Recent studies show that externally applied electrical stimulus can influence cell activity, increased proliferation, differentiation, and extracellular matrix production. Numerous clinical interventions demonstrate the use of electrical stimulation (ES) in cardiac pacemakers, transcutaneous electronic nerve stimulators (TENS) devices, chronic wound dressing products, and so on. For advancing further ES therapies, it is therefore pivotal to study the underlying signalling pathways that play a vital role in cell and tissue development. In this project, we investigate plausible lineage specifications that human mesenchymal stem cells (hMSCs) can attain when stimulated with different electrical regimes. Compared to the significance of stem cells in tissue engineering applications, only a few studies have investigated the role of biologically relevant ES regimes for stem cell lineage specification.

METHODS: Electrical stimulation is applied using a newly designed 12-well plate gold-coated stainless steel electrical stimulation bioreactor for *in vitro* bone marrow-derived hMSC proliferation and differentiation. Using this bioreactor, we anticipate determining the appropriate electrical regimes which can direct lineage specification in hMSCs by varying electric field intensities between 50-150 mV/mm.

RESULTS: Preliminary results show promising cell proliferation and differentiation of mesenchymal stem cells towards osteogenic lineage when stimulated with direct current ES of 100 mV/mm (stimulated 1h/day for 7 days) and osteogenic differentiation conditions.

DISCUSSION & CONCLUSIONS: The custom-designed 12-well plate ES bioreactor shows promising applications for *in vitro* high throughput ES of cells. Direct DC voltage of 100 mV/mm induced positive effects on osteogenic differentiation as various cell proliferation assays performed on hMSCs showed enhanced metabolic activity. Further lineages specifications of hMSCs, viz. adipogenic and myogenic, and their gene expression during differentiation are currently investigated across voltage regimes between 50-150 mV/mm and waveforms.

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Keywords: Biomaterials, Bioreactors

PP-034

Gelatin and hydroxyapatite derived 3D printed scaffold towards osteogenic regeneration

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INTRODUCTION: Tissue model mimicking bone like material was fabricated and characterized using gelatin (gel)-hydroxyapatite (Hap) derived bioink. Herein, we prepared the bioink by varying the ratio of Gel and Hap, a key organic component of native bone ECM (extracellular matrix).

METHODS: Gel and HAP based bioink was formulated at optimum concentrations for fabricating 3D printed substrate by extrusion-based 3D printing. Material characterization such as XRD, FTIR and Raman spectroscopic were performed for confirming the presence of chemical compositions. Further study with mechanical and invitro degradation study demonstrated the mechanical stability of the printed substrate. The in-vitro study with MSCs showed future opportunities and potential of printed scaffolds for bone regeneration application.

RESULTS: FTIR and Raman spectroscopic analysis were done to characterize chemical constituents and degree of crosslinking. Rheological studies were performed to inspect shear thinning property along with degree and time period for crosslinking. Young's modulus of scaffold is 77 MPa by compressive mechanical testing and 63% swelling ratio in 72h (PBS, 37°C). Microstructure analysis and scaffold porosity was determined by SEM, AFM and surface profilometer study. In vitro cytotoxicity study was performed by MTT, live-dead assay and rhodamine phalloidin-DAPI analysis resulted with 110% cell viability and enhanced cell to cell interaction.

DISCUSSION & CONCLUSIONS: Bioink has thinning behaviour and optimum gelation period allowed extrusion and prevent gravity flow following strut deposition. The printed scaffolds with optimal porosity supported cell proliferation and migration. In vitro cytotoxicity assays exhibit high cellular proliferation and growth with presence of cell-to-cell interaction. Differentiation studies exhibit enhanced osteogenesis of MSCs with mineralized matrix deposition characterized by gene expression of Runx2, OCN and BMP2. Mineralised bone nodules were qualitatively characterised by positive staining of Alizarin Red. The fabricated substrate proved to be a suitable material and had potential for generating bone-like material by inducing osteogenic differentiation.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: 3D printing and bioprinting, Biomaterials

PP-035

Preparation of multifunctional porous biocomposites made of chitosan, bioglass and peptides

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INTRODUCTION: Polymer/ceramic porous composites are the materials most often considered by researchers in last years in terms of their use as scaffolds for regenerating bone tissue. Adequate porous structure of such materials allows cell migration and ensure nutrient flow in their use as implants. Various methods of obtaining porous materials are known from the literature, such as simple lyophilization or electrospinning. Depending on the method used, it is possible to obtain biomaterials with different properties.

The materials used to make porous scaffolds for medical purposes should be biocompatible, biodegradable and bioactive [1]. Such properties are exhibited by for example, chitosan biocomposites with the addition of bioglass that increases the bioactivity and could allow antibacterial activity. Additionally in order to extend the properties of composites they could be also enriched by different additions, like biologically active peptides for example with pro-regenerative, anti-inflammatory or antibacterial properties.

The main difficulty with porous polymer composites is their low durability, rapid degradation and poor mechanical properties. In the case of chitosan scaffolds this can be prevented by crosslinking or stabilizing with appropriate methods and agents [2].

Both the choice of the method of introducing active substances into the composite and the method of obtaining the porous structure and the conditions for stabilizing the scaffold are therefore key issues in the design and preparation of new porous chitosan-bioglass-peptide scaffolds.

METHODS: The porous structure of chitosan-bioglass-peptide scaffolds was obtained by freeze-drying method. The introduction of peptides into porous composites at the stage of forming a stable dispersion and by applying them to the surface of the finished structure are discussed. Six methods of crosslinking/stabilization of chitosan composites were used.

RESULTS: In the poster, ways of forming multifunctional scaffolds by freeze-drying are presented. The introduction of peptides into porous chitosan-bioglass composites and methods of crosslinking/stabilization of chitosan composites are discussed. In all of the used methods we obtained stable porous composites with appropriate microstructure, enriched with active peptides.

DISCUSSION & CONCLUSIONS: All the methods presented in the poster for introducing bioactive components, crosslinking/stabilizing the structures were successfully applied.

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Keywords: Composite materials, Biomaterials

PP-036

Comparison of magnetic nanoparticle activation of mechanotransduction via antibody and aptamer

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INTRODUCTION: Magnetic particle (MNP) based technology platforms use superparamagnetic nanoparticles conjugated with targeting biomolecules to apply mechanical force directly to mechanoreceptors on the cell surface, stimulating mechanotransduction and downstream signalling. This technique has been reported to promote differentiation toward musculoskeletal cell types from adult stem cells from multiple sources[1,2].

METHODS: Aptamers are short nucleic acid sequences capable of specific, high-affinity molecular binding which bind to proteins and receptors with a higher affinity compared to antibodies. Aptamers have several advantages, including small size, good biocompatibility, and low immunogenicity[3]. In this presentation, we compare the agonists for binding to mechanoreceptors using MNP-platforms. The MNPs were conjugated with Cd49e antibody (Thermo Fisher) for binding to the integrin $\alpha 5$, and aptamer (Aptamer Science) for binding to the integrin $\alpha 5 \beta 1$ receptors. We compared the impact of mechano-activation of receptors on the Wnt signalling pathway and osteogenesis via the conjugated magnetic nanoparticle stimulation and magnetic field.

RESULTS: The genetic responses of cells mechano-activated by the conjugated MNPs were studied by using TCF/LEF reporter cell line and gene expression of RUNX2 in human mesenchymal stem cells (MSCs). Analysis of data from the reporter cell line demonstrated that mechano-activation of the cells by MNPs conjugated with aptamer resulted in significant upregulation of the Wnt signaling pathway relative to the activation of the cells by MNPs conjugated with the antibody. Furthermore, the mechano-activation of MSCs by the MNP-aptamers significantly upregulated the expression of RUNX2 compared to the mechano-activation of MSCs by the MNP-antibodies that can improve osteogenesis.

DISCUSSION & CONCLUSIONS: In this study, we compared the mechano-activation of cells via MNPs conjugated with CD49e antibodies and aptamers which can bind to integrins. The data demonstrated that mechano-activation of cells by MNP-aptamers significantly enhances upregulation of the RUNX2 and LEF1 genes compared to using MNP-antibodies which can potentially promote bone regeneration.

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Keywords: Biomaterials, Nanomaterials (inc graphene)

PP-037

Lightweight, high strength bioactive polysaccharide-based scaffold with potential to treat bone tumors

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INTRODUCTION: Bone tumors, especially those in osteosarcoma, are especially prevalent in children and adolescents with high mortality rate. Despite the recent advances, the standard clinical treatments such as chemotherapy, radiation, and resection surgery fail to completely remove the tumor, and therefore postoperative recurrence and metastasis are often observed. In addition, the large areas of bone removed in surgeries cannot repair itself, negatively affecting the quality of life of patients. Thus far, finding biomaterials to be used for tumor therapy simultaneously with bone regeneration remains a clinical challenge.

METHODS: In this context, porous multifunctional platforms can be leveraged to produce bioactive scaffolds that allow enhanced repair of the surrounding tissue, while acting as long-term localized therapy against cancer. Herein, gallium-doped bioactive glass (Ga-BG) and curcumin-carrying nanoparticles based on polylactic-co-glycolic acid based (PLGA-NPs) were first prepared by sol-gel synthesis and single emulsion-solvent evaporation method, respectively. Then, such particles were added to cellulose nanofibrils (CNF), producing highly porous multifunctional structures via freeze-drying.

RESULTS: As result, the produced flexible, yet strong material (compression strength of about 24 ± 1 kPa) presented the potential to combine the required features for bone cancer treatment. Specifically, the total porosity more than 50% of CNF-based structure allows the nutrients transport and cell functions and, consequently, assisting bone regeneration. The addition of Ga-BG into CNF increased the bioactivity of the cellulose nanostructure due to hydroxyapatite formation, proved by the band at 1030 cm^{-1} (crosslinked $\equiv\text{Si-O-Si}\equiv$) from infrared (FTIR) spectrum. Gallium ions (Ga^{3+}) released from Ga-BG are able to selectively killed osteosarcoma cells through competition between abiogenic and biogenic ions in the blood, while Na, Ca, Si and P species improved the cell differentiation. Finally, the drug carried in PLGA-NPs was successfully released from of the swollen scaffolds, proving the possibility of therapeutic payload delivery.

DISCUSSION & CONCLUSIONS: In summary, the presented strategy to explore the synergy of organic-inorganic materials preparing multifunctional platforms has potential to open up new horizons for bone cancer treatment. Moreover, the simplicity, scalability and versatility of the proposed method can inspire the design of other functional material for regenerative medicine.

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REFERENCES:

Keywords: Composite materials, Polymers - natural / synthetic / responsive

PP-038

The effect of molecular weight on the thermal processability of novel L-alanine based poly(ester amide)s

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INTRODUCTION: α -Amino acid based poly(ester amide)s (AAA-PEAs), combining biocompatibility and biodegradability of polyesters with the superior mechanical properties of polyamides, are polymers whose importance in the biomedical field has increased considerably in recent years. The main advantage of these materials is the versatility of the structures obtained, allowing fine-tuning of physicochemical properties (e.g., hydrophilicity, stiffness, degradability). The presence of α -amino acids enables the enhancement of cell-material interactions. Despite the advantages and properties of AAA-PEAs, their thermal processing, which could open new avenues in additive manufacturing for tissue engineering and regenerative medicine, has not yet been extensively explored. In this work, we propose the synthesis of AAA-PEAs based on L-alanine (PEA-ala), L-alanine/glycine (PEA-ala-gly (75:25)) and L-alanine/glycine/jeffamine (PEA-ala-gly-jeff (50:25:25)) and we have investigated the effects of their molecular weight on their thermal processability. The AAAs were selected based on the envisaged applications for these AAA-PEAs, which is the regeneration of articular cartilage(AC). Glycine and L-alanine are present in greater amounts in the structure of collagen, which is the most abundant protein in AC. Jeffamine (jeff) was used as co-monomer to increase the hydrophilicity of the PEAs.

METHODS: The AAA-PEAs were prepared by solution polycondensation. The PEAs were subsequently hot pressed to prepare films. These were tested in phosphate buffered saline(PBS) (pH=7.4) at 37 °C for swelling ability(24h) and in vitro degradability(4 weeks).

RESULTS: All PEAs were successfully synthesized. The molecular weight ranged from 30kDa to 80kDa, with polydispersities close to 1.50. Only the AAA-PEAs with molecular weight higher than 50kDa were able to produce non-brittle and flexible films. The Tg of PEA-ala and PEA-ala-gly was found to be approx. 15oC, and with the additon of jeff the value decreased to -14 oC. PEA-ala and PEA-ala-gly films showed a maximum swelling capacity close to 3%, after 4 hours of incubation in PBS(pH=7.4), whilst PEA-ala-gly-jeff films showed a significant increase in swelling capacity(15%) under the same conditions. In vitro degradation tests revealed an increase in mass loss of the PEAs from 2% to almost 6% over 4 weeks, with the additon of jeff.

DISCUSSION & CONCLUSIONS: This work allowed to determine the minimum molecular weight of AAA-PEAs required to obtain high quality films(non-brittle and flexible) by thermal processing, information that could be used for further studies in 3D printing. Furthermore, it was shown that the properties of AAA-PEAs-based films can be easily modified by changing their structure.

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Keywords: Biomaterials,

PP-039

Effect of a biodegradable poly(DL-lactide-co- ϵ -caprolactone) mesh loaded with or without antifibrotics in comparison with a commercially available polypropylene mesh *in vitro* and *in vivo* in a subcutaneous rabbit model

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INTRODUCTION: Wound healing is a complex process in response to the opening of a tissue and the absence of cellular structures. To facilitate healing and prevent infection, the affected area is usually covered with a sterile dressing.¹ Nowadays, commercially available meshes are made of non-immunogenic and non-toxic, physically and chemically inert materials like polypropylene (PP). However, in some patients, they can trigger inflammation, fibrosis or calcification processes.² To avoid this problem, we have developed a drug delivery system based on biodegradable poly(DL-lactide-co- ϵ -caprolactone) (PLCL) electrospun meshes. To increase the healing procedure, we also loaded our biodegradable mesh with two different antifibrotics, pirfenidone (PIRF) and triamcinolone acetonide (TA) and studied their effect both *in vitro* and *in vivo* in a subcutaneous epithelial rabbit model.

METHODS: PLCL electrospun meshes were fabricated, loading two antifibrotics: PIRF and TA. Physico-chemical characterization was carried out, conducting hydrolytic degradation, drug release and scanning electron microscope (SEM) studies. For *in vitro* functional analysis, both cytotoxicity and inflammation evaluation (interleukin 6 (IL-6) release) were studied on RAW 264.7 macrophages. Finally, *in vivo* studies were performed in a subcutaneous rabbit model following ISO 10993-6, where irritation, fibrosis and neovascularization indexes were compared with commercial PP meshes.

RESULTS: PLCL meshes were fabricated without aggregates. All meshes showed complete degradation after 120 days at 37 °C and both antifibrotics showed high values of cellular metabolic activity. Antifibrotic inflammation showed lower IL-6 release than that observed on the positive control with lipopolysaccharides (LPS) activation of the macrophages. After 72 hours in contact of PLCL meshes with the cells, IL-6 release was significantly lower in antifibrotic-loaded systems. *In vivo* evaluation showed high irritation and fibrosis indexes after 2 weeks in the controls and a significant decrease only in PLCL meshes after 4 weeks. In antifibrotic-loaded meshes, 2.5% TA and PIRF meshes showed the lowest fibrosis and irritation indexes.

DISCUSSION & CONCLUSIONS: 4 weeks after implantation, PLCL meshes improved the irritation index and the presence of fibrosis compared to commercial PP. This improvement was superior even to meshes that incorporated both antifibrotics, making our PLCL meshes a real alternative to commercially available meshes, even without any bioactive molecule.

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Keywords: Polymers - natural / synthetic / responsive, Drug delivery

PP-040

Application of a novel 3D porous Poly-epsilon-lysine hydrogel, Trabeculate™, to create a biomimetic structure of the trabecular meshwork of the eye.

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INTRODUCTION: Glaucoma is a leading cause of irreversible blindness worldwide. In primary open angle glaucoma (POAG), elevated intraocular pressure (IOP) damages retinal nerves as a result of changes to the trabecular meshwork (TM) of the eye. The TM is a complex 3D porous tissue located in the anterior chamber, responsible for filtration and regulation of aqueous humour outflow (AHO). It consists of large overlapping extracellular matrix beams lined with phagocytic TM cells and spaces between these beams form a porosity gradient from the Uveal meshwork, to the Juxtacanalicular tissue, from 40-9µm, respectively. In POAG, the TM becomes stiffer, pores are obstructed and cell numbers deplete, creating AHO resistance. Previous research has focused on 2D culture models which do not reflect the TM environment. We have therefore studied the suitability of novel poly-ε-lysine (PeK) based hydrogels, with tuneable pore sizes and stiffness capabilities in both 2D and 3D, on TM cell growth and function.

METHODS: 2D PeK gels were chemically cross linked (CL) to 60, 70 or 80% CL to determine their biocompatibility with immortalised TM cell lines (NTM5) and influence on cell phenotype. Stiffness of the 2D gels was measured on the Univert. A commercially sensitive porogen was used to create porous 3D gels. Gels were freeze-dried before SEM imaging to measure pore sizes. NTM5 were labelled with DiD labelling solution for live imaging and gels were cryosectioned over 7 days to examine cell morphology and infiltration using immune fluorescent staining. Cell metabolism was measured using resazurin.

RESULTS: On 2D gels NTM5 attached to the gel surface on day 1 and proliferated over 7 days. Cells on 60% CL gels formed spheres, whilst cells on 70-80% cross linked gels formed monolayers with cobblestone morphology. Mechanical testing revealed that the compressive and tensile moduli ranged between 0.5-1.5 MPa and 100kPa - 400kPa, respectively. On 3D gels, 30-50µm pores were observed using SEM. NTM5 attached and over 7 days and had infiltrated the 3D gels, and metabolism was 2-fold higher than on normal tissue culture plastic after 4 days.

DISCUSSION & CONCLUSIONS: NTM5 cells attach and proliferate on porous and non-porous PeK hydrogels. Pore and beam sizes in the 3D gel closely resemble that of the TM. For 2D 60-80% CL gels, mechanical properties were similar to that of glaucomatous TM tissue, making them a suitable model for glaucomatous TM tissue.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: Eye, Hydrogels and injectable systems

PP-041

Hyaluronic acid-tyramine bioink for coaxial bioprinting of cell-laden filaments

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INTRODUCTION: The demand for tissue and organ replacements is increasing globally. Tissue engineering and regenerative medicine have been introduced to restore these damaged tissues to functional ones with aid of 3D bioprinting. Bioinks for 3D bioprinting should have good printability, biocompatibility, and high shape fidelity after printing. From this perspective, we focused on developing a high molecular weight (HMW) hyaluronic acid-tyramine (HA-TA) bioink to coaxially print cell-laden filaments.

METHODS: HMW HA-TA was synthesized in which the tyramine moieties can be crosslinked by a mild enzymatic reaction in the presence of horseradish peroxidase (HRP) and H₂O₂. HMW HA-TA with HRP was coaxially extruded, with Pluronic F127 including H₂O₂ as a supporting outer shell. The HA-TA in the inner core was near-instantaneously crosslinked by diffusion of H₂O₂, yielding stable HA-TA filaments. Similarly, cell-laden filaments were produced by combining bovine primary chondrocytes or fibroblasts with the biomaterial. The extruded cell-laden filaments were cultured for up to 7 days in vitro. In addition, the physical properties such as rheological properties, swelling ratio, and degradation of the biomaterial were examined.

RESULTS: HMW HA-TA was synthesized successfully through DMTMM-mediated amidation. The functional biomaterial was assessed and proven through the formation of gels upon HRP-mediated crosslinking of the phenolic moieties. Hydrogel formation of HMW HA-TA at 3 different percentages (1.3, 1.8, or 2.2 %w/v) and with HRP (5.5 U/ml) was near instantaneous. For chondrocyte- and fibroblast-laden filaments, over 90% and 80% of the cells were viable in all conditions at day 0 respectively. Over time, the viability of chondrocytes and fibroblasts decreased to about 82% and 67% respectively for 1.3 %w/v HMW HA-TA on Day 7, while the viability of chondrocytes in higher HMW HA-TA concentrations had lower cell viability. The width of non-cell-laden filaments for each polymer percentage (1.3, 1.8, or 2.2 %w/v) increased from 812 μm $\pm$ 34 to 1458 μm $\pm$ 111 with an increase in the IC pressure and fixed OS pressure.

For physical properties, the swelling ratio decreased with an increase in the DS and increased with an increase in polymer concentration. All HMW HA-TA hydrogel samples were degraded completely within 15 days in 2.5 U/ml hyaluronidase. The degradation rate was inversely proportional to the concentration and DS.

DISCUSSION & CONCLUSIONS: The development of an instantaneously crosslinking HMW HA-TA bioink for the production of filaments by coaxial bioprinting was successful and can be used for tissue regeneration in the future.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: Biomaterials, 3D printing and bioprinting

PP-042

Chondrogenesis is improved via dynamic mechanical stimulation in ATDC5 embedded agar hydrogels

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INTRODUCTION: Osteoarthritis (OA) is a prevalent age-related joint disease affecting millions worldwide. There is currently no cure for OA and understanding the disease mechanisms is crucial for discovering potential therapeutics. Hydrogels are a promising tool in the effort to engineer biomimetic tissue for in vitro studies of articular cartilage diseases. Mechanical loading has previously been shown to improve chondrogenesis in hydrogel encapsulated cells. We embedded ATDC5 cells in agar hydrogels, stimulated them with cyclic compressive loading mimicking physiological loads and assessed the effect on cell viability, differentiation, and extracellular matrix (ECM) production.

METHODS: 106 ATDC5 cells/ml were encapsulated in 2% v/w agar and the hydrogels were compressed at day 7 with four (sinusoid, triangle, square, sawtooth) compression regimes at 0.33Hz. The sinusoid regime was taken further for 14 days at 0.33Hz and 1Hz. TUNEL assay and qPCR were used to quantify cell apoptosis and gene expression respectively. RNA sequencing analysis was performed on 0.33Hz compressed samples. Scanning electron microscopy and histology were used to assess ECM deposition. Atomic force microscopy was used to assess mechanical properties.

RESULTS: Cell death increased in all regimes, with sinusoidal compression resulting in the lowest and sawtooth the highest. Col1a1 was significantly upregulated following triangle compression, while Col2a1 was downregulated in square and sawtooth. Cells observed at 21 days in 0.33Hz and 1Hz compressed samples showed ECM development with adherence to the hydrogel surface. Nanoscale Young's modulus increased after 21 days for both compressed and uncompressed samples; however no significant differences were observed between the two. The transcriptomic data showed increased chondrogenesis and a decrease in cartilage catabolic genes.

DISCUSSION & CONCLUSIONS: We demonstrate that non-physiological regimes upregulate dedifferentiation (col1a1) and downregulate chondrogenesis (col2a1). This may be due to these regimes not allowing proper relaxation of the cells between force application. ECM production and increased nanoscale mechanical properties observed after 21 days at 0.33Hz and 1Hz sinusoidal compression suggest that physiological loading increases ECM deposition and in turn increases the stiffness at nanoscale levels, whilst the transcriptomic data suggests that physiological loading regimes may in fact be chondroprotective. Future studies will focus on human chondrocytes to assess the impact of mechanical stimulation on anabolic and catabolic responses in tissue engineered cartilage.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: Hydrogels and injectable systems, Cartilage / joint and arthritic conditions

PP-043

Comparing the cytotoxicity and crosslinking efficiency of three commonly used photo-initiators when making cell-laden hydrogels for biomedical applications

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INTRODUCTION: Biomedical hydrogels that undergo a rapid liquid-to-solid phase transition in the presence of cells and tissues often use a photopolymerization strategy to cross-link the polymer network. There are only a few photoinitiators (PIs) that are commonly used for crosslinking biomedical hydrogels, including Irgacure2959 (I2959), lithium phenyl-2,4,6-trimethylbenzoylphosphine (LAP) and EosinY (EoY). These PIs are illuminated at 365nm, 405nm and 525nm, respectively. The photopolymerization conditions, including PI concentration and illumination intensity may affect the crosslinking and the cytotoxicity of the reaction. Herein we tested the three PIs to optimize the maximum crosslinking efficiency while minimizing cell death associated with the reaction.

METHODS: PEG-fibrinogen (PF) hydrogels were crosslinked by photopolymerization using the three PIs. The maximum shear storage modulus (G' max) of the hydrogels and the crosslinking reaction kinetics were assessed by rheological time-sweep measurements. Neonatal human dermal fibroblasts (NHDF) were encapsulated in PF hydrogels during photopolymerization, and their viability and morphology were evaluated after 2 and 24 hours using a Calcein/Ethidium Live/Dead assay and Trypan-Blue exclusion assay. The crosslinking and viability data were quantitatively compared for the different PIs, PI concentrations, and illumination intensities.

RESULTS: Optimized parameters were reported for each PI in terms of concentration and illumination intensities. The optimized conditions for photopolymerization of both I2959 and EoY resulted in hydrogels with a similar G' max value of ~280 Pa, whereas optimal LAP photoinitiation produced hydrogels with G' max of ~200 Pa. Cell viability at the maximal concentration and illumination conditions was above 90% for I2959 and EoY, whereas the viability was 80% with the LAP; However, decreasing the LAP concentration from 1% to 0.1% resulted in 90% viability of the cells without compromising the crosslinking efficiency.

DISCUSSION & CONCLUSIONS: All PIs had an optimal concentration and illumination intensity for producing hydrogels with a distinct G' max value, indicating optimal crosslinking conditions were achievable for each PI. The PIs had significantly different crosslinking kinetics, which were affected by concentration and illumination intensities. Despite this, the final PF hydrogel G' max values were similar for I2959 and EoY, whereas the LAP produced hydrogel that exhibited significantly lower values of G' max, signifying a 30% reduction in the LAP's crosslinking efficiency. In addition, changes in LAP concentration had a more profound impact on cell viability when compared to EoY or I2959. Taken together, these data suggests that LAP may be less effective for crosslinking hydrogels, and more cytotoxic when compared to the other alternative PI systems.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

PP-044

Peptide Hydrogels for Local Drug Delivery

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INTRODUCTION: Drug delivery remains one of the most important aspects of disease treatment and directly affects patient outcomes. Systemic drug delivery is commonly used due to ease of administration through routes such as oral or intravenous. However, not all of the drug reaches the desired target in the body and there is the potential for off-target side effects. In addition, repeat administration is required to maintain levels in the therapeutic range. Local drug delivery has emerged as a promising alternative to overcome these challenges as it can target therapeutics at the intended tissue and maintain a desirable dose for a longer period of time¹. This research aims to develop peptide hydrogels as a drug delivery system to provide more targeted and localised delivery of large molecule therapeutics such as anti-VEGFs or proteins. Self-assembling peptide hydrogels are biomaterials that can be designed as injectable, biocompatible and biodegradable. They are capable of trapping molecules, such as drugs, within their cross-linked polymer network and slowly releasing them over time².

METHODS: Mechanical properties will be characterised by rheology to determine whether anti-VEGF loading disrupts self-assembly of the peptide hydrogel network. Using UV-Vis spectroscopy the concentration of anti-VEGF released from the hydrogel over time will be quantified to assess the release kinetics, specifically the nature of burst release and sustained release profiles.

Biocompatibility of the hydrogels will be tested with a relevant cell line in 3D cell culture, cell death and proliferation will be quantified using Live/Dead, Pico Green and BrdU assays.

RESULTS: We will show by rheological measurements that mechanical properties of the hydrogels can be altered by adjusting peptide concentration and fiber-fiber interactions. As elucidated, we will use UV-Vis spectroscopy to demonstrate that changing the primary peptide sequence influences drug-hydrogel interactions, and therefore burst and sustained release profiles. These interactions, such as electrostatic attraction, primarily control release kinetics through diffusion and network degradation.

DISCUSSION & CONCLUSIONS: Overall, we will demonstrate the potential of peptide hydrogels as a drug delivery vehicle for more targeted and temporal delivery of therapeutics.

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Keywords: Drug delivery,

PP-045

Bioink Based On Decellularised Extracellular Matrix For Bone Graft Bioprinting

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INTRODUCTION: Bone tissue regeneration remains one of the great challenges in regenerative medicine. Despite the many existing techniques using different types of biomaterials for the repair of bone tissue injuries, a fully functional cure has not yet been found because of the complexity of mimicking the bone microenvironment¹. The use of decellularised extracellular matrix (dECM)-based bioinks aims to fill these gaps by preserving and maintaining the structural and biochemical interactions of the native environment². Therefore, the aim of this study is to generate a novel biomimetic bioink based on dECM obtained from mesenchymal stem cells (MSCs) for bone tissue engineering (BTE).

METHODS: MSCs from lipoaspirate obtained from various healthy donors were isolated and grown under good laboratory practices (GLP) conditions. To generate a hydrogel bioink for BTE, bone-dECM was obtained from MSCs grown under a combination of differentiation factors and prepared by proper decellularization procedures. The bone-dECM was characterised in vitro by measuring the DNA content, immunofluorescence and histological techniques assays. To allow the generation of a novel 3D bioprintable bioink, it was formulated by the addition of bone-dECM to a combination of natural and synthetic biomimetic materials. The bioink was subsequently evaluated by assessing rheological properties, cell viability, and biocompatibility assays.

RESULTS: After obtaining the MSCs-derived extracellular matrix, its osteogenic differentiation and correct decellularisation were confirmed. In addition, the bone-dECMs showed excellent biochemical properties. Rheological tests confirmed that our bone-dECM-based bioink loaded with MSCs possesses mechanical viscoelastic properties suitable for use as an injectable hydrogel. In vitro studies revealed high cell viability and proliferation after 10 days in standard cell culture conditions after 3D bioprinting.

DISCUSSION & CONCLUSIONS: Bone-dECMs show good biocompatibility and biochemical properties like those of native bone tissue. Our results indicate that bone-dECM is a good, easily adaptable, and low-cost biomaterial for use in bone regenerative medicine and can be used in combination with other biomaterials for their printability. These findings demonstrate for the first time the potential of

this bone-dECM-based bioink to be applied as an implant to promote bone repair and regeneration.

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Keywords: Decellularised matrices, 3D printing and bioprinting

PP-046

Low-intensity pulsed ultrasound is effective in counteracting inflammation and inducing chondrogenic differentiation of adipose-stromal cells in 3D piezoelectric hydrogels

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INTRODUCTION: A major challenge in cartilage tissue engineering is to develop hydrogels capable of providing an instructive biomimetic environment to effectively drive mesenchymal stromal cells (MSCs) differentiation and able to counteract inflammation [1].

Inflammation plays a key role in the progression of OA, and this condition can affect the viability and transcriptional activities of the tissue regeneration process [2]. Low-intensity pulsed ultrasound (LIPUS) and piezoelectric nanomaterials represent an interesting tool for inducing chondrogenesis [3-4]. The aim of this study was to evaluate the effects of LIPUS in counteracting inflammatory milieu and positively committing the chondrogenesis of encapsulated human adipose stromal cells (hASCs) in 3D piezoelectric hydrogels.

METHODS: hASC (2*10⁶ cells/mL) were embedded in 3D VitroGel RGD® hydrogel with or without nanoparticles (GO, 25µg/ml, BaTiO₃, 50µg/ml) and exposed to specific LIPUS stimulation every 2 days until day 10. Hydrogels were cultured and analysed in basal condition (day 2) and after chondrogenic commitment at days 10 and 28. At each time point cell viability, cytotoxicity, gene expression and immunohistochemistry of COL2, ACAN, SOX9, and COL1, and the release of the inflammatory cytokines IL6 and IL8 were evaluated. To mimic acute and chronic inflammatory conditions we added IL1β (10 ng/ml) 36 hours before starting the chondrogenic commitment (acute) or along all the chondrogenic induction phase from day 2 to day 28 (chronic). Human CD14⁺ macrophages derived from buffy coat were used as positive control to confirm LIPUS anti-inflammatory effects.

RESULTS: In all 3D hydrogels we evidenced that LIPUS treatment did not affect negatively the viability and cytotoxicity of the embedded cells. On day 10, in both hydrogel formulations, LIPUS treatment was effective in decreasing IL6 and IL8 release both in the acute and chronic groups. Interestingly, at day 28 we evidenced only in acute group a significant increase of the chondrogenic markers in 3D piezoelectric hydrogel treated with LIPUS associated to a decrease of inflammatory cytokines. By contrast, at day 28 since in the chronic group, the cytokines were still very high, the COL2A1 expression was undetectable.

DISCUSSION & CONCLUSIONS: Overall, these data confirmed that the continuous LIPUS treatment was efficient in counteracting acute and chronic inflammation (known to have a key role in the pathogenesis of osteoarthritis). However, only when the inflammatory cytokines were switched-off as occurred in acute group, the chondrogenic commitment of encapsulated-hASCs in 3D piezoelectric hydrogel was induced.

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Keywords: Hydrogels and injectable systems, Multipotent (mesenchymal) stem cells

PP-047

Novel Synthesis of Covalently Crosslinked Organosilane Functionalised Gelatin-Based Organic-Inorganic Hybrid Hydrogels

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INTRODUCTION: Hydrogels have received considerable attention for use as a suitable scaffold material for tissue engineering. However, their poor long-term mechanical properties limit their use (1,2). Polycondensation of an inorganic co-network via sol-gel reaction to form an organic/inorganic hybrid is a strategy to improve hydrogel physical properties resulting from synergy of the properties of the two components (3,4). This study optimises novel synthesis routes for producing Class II covalently crosslinked gelatin-based-silica hybrid hydrogels.

METHODS: Gelatin and gelatin derivatives provided by ClaroTM (a brand of Tessenderlo Group) were modified with various organosilane crosslinkers. Gelatin methacryloyl (GelMA) and gelatin norbornene (GelNB) were covalently crosslinked with organosilane epoxides and thiols, respectively. Reaction conditions were studied to optimise crosslinking degree using NMR and crosslinking quantification assays. Various formulations of hybrid hydrogels were made with silane functionalised gelatin-derivatives (organic) and colloidal silica (inorganic). Hydrogels were observed for homogeneity and gel time, and selected hydrogels were further characterised by investigating their mechanical and biological properties.

RESULTS: Conditions (temperature, pH and solvents) were identified to extend functionalisation time leading to increased coupling of organosilane crosslinkers to various gelatin-derivatives. Numerous organic:inorganic hybrid ratios (60:40, 40:60, and 20:80) were synthesised and tested with the 60:40 formulation undergoing phase separation and no gelation. Additionally, hydrogels with organosilane crosslinkers, with un-coupled crosslinker removed, did not show superior properties with respect to degradation compared to those without a crosslinker.

DISCUSSION & CONCLUSIONS: There is much confusion and disagreement in literature with regards to functionalisation of gelatin with organosilanes and the optimum conditions to employ. This has resulted in hybrids that may have no crosslinking, or very little, between the organic and inorganic and so diverse and unpredictable properties. When un-coupled organosilane crosslinker is removed post-functionalisation, no difference in degradation of hybrids in media between hydrogels with and without crosslinking is observed. It can be suggested that the excess crosslinker within the hybrid influences the properties but not in intended manner. Furthermore, this study reveals various novel ways to overcome the issues lack of functionalisation and different hybrid formulations that result in variation of mechanical properties.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

PP-048

Bioengineering stromal cornea using fish collagen electrospun membranes

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INTRODUCTION: Corneal disease is a main cause of vision impairment and blindness, and the only effective treatment is corneal transplantation, which has associated risks (i.e., shortage of compatible donor, infection, ethnic constraints, and high costs). Other approaches such as the use of artificial corneas made of synthetic polymers have been used; however the risk of graft rejection is present. Tissue Engineering and Regenerative Medicine (TERM) are offering promising approaches, such as the use of amniotic membranes, decellularized corneas or cell-based therapies, although none of them have reached yet the features needed to promote cornea regeneration. Strategies such as electrospinning have potential to mimic the natural form of extracellular matrix of cornea, particularly when using a native substrate of cornea such as collagen.

METHODS: The present work explored marine origin collagen and gelatin as building blocks to produce bioengineered membranes using electrospinning technique and further evaluating their in vitro performance for corneal stromal regenerative application. Collagen and gelatin isolated from by-products of fish processing industries, particularly blue shark (*Prionace glauca*) and codfish (*Gadus morhua*) skins, were used to produce pure polymeric solutions, electrospun to generate nanofibrous meshes. The resulting membranes were crosslinked with glutaraldehyde vapor and characterized regarding the chemical and physical (morphological, structural, optical, mechanical, permeability) properties, as well as its biocompatibility using human keratocytes.

RESULTS: The results demonstrated the production of porous fibrous membranes with interconnected pores and mechanical properties very similar to the human cornea. Moreover, the membranes exhibited also an hydrophilic character and permeability to solute as required for the nutrition of corneal stromal tissue. Membranes produced using gelatin from shark and codfish skins presented superior potential to be used as corneal stromal regeneration candidates since they combine good optical results (light transmission above 80%) with excellent biocompatibility, as shown by high cell viability and proliferation.

DISCUSSION & CONCLUSIONS: These results suggested that collagenous materials isolated from fish by-products have great potential for biomedical field, namely in the production of electrospun membranes for corneal stromal regenerative applications, contributing not only for biotechnological valorization of fish by-products but also to establish new building blocks and biomaterials for

biomedical applications, namely in tissue engineering.

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Keywords: Polymers - natural / synthetic / responsive, Eye

PP-049

Micropatterned glycopeptide-based supramolecular hydrogels promote the alignment and contractility of cardiomyocytes

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INTRODUCTION: Cardiac tissue regeneration is still a challenge in tissue engineering and regenerative medicine. A functional recovery of the cardiac tissue requires an adequate contractility of exogenous and/or endogenous cardiomyocytes.^{1–2} Different biomaterials can be used to induce such contractility. Herein, we propose the use of glycopeptide supramolecular gels. Due to the non-covalent nature of the supramolecular interactions (e.g. π - π , CH- π), it is possible to generate dynamic systems³ that respond to different (bio)chemical stimuli. Moreover, these hydrogels can be micropatterned in order to induce the alignment of cardiomyocytes as in cardiac tissue.

Glycopeptides copycat the glycoproteins of the ECM and thus, are closer ECM mimic at biofunctional term.⁴

METHODS: The glycopeptide Fmoc-FF-GlcN6S was synthesized using the DCC-NHS coupling strategy to conjugate glucosamine-6-sulfate (GlcN6S) to fmoc-diphenylalanine (Fmoc-FF). A pre-gelation solution was prepared by temperature switch method. The morphology of the generated nanofibers and the presence of the micropatterns were evaluated using the SEM and AFM. Electrochemical impedance spectroscopy (EIS) was also used to assess the effect of glycosylation on the propagation of electrical stimuli. Cardiomyocytes were cultured in the micropatterned hydrogels and their viability and morphology was assessed. Expression of specific proteins was also evaluated by immunostaining and Western blot analysis.

RESULTS: SEM showed that the hydrogel is formed by entangled nanofibers. AFM demonstrated that micropatterning was transferred with high fidelity to the hydrogel surface. The EIS measurements evidenced a higher conductivity of the Fmoc-FF-GlcN6S hydrogels when compared to Matrigel (control). Cardiomyocytes (CMs) cultured on Fmoc-FF-GlcN6S hydrogels were viable (Lactate Dehydrogenase (LDH) and Live/Dead data similar to the control, TCPS). The cytoskeleton visualization (Phalloidin staining) demonstrated that cells were aligned along the patterns. Immunostaining and WB for cardiomyocytes specific markers TroponinT and Phospholamban demonstrated comparable expression in the hydrogels and in the TCPS for the selected.

DISCUSSION & CONCLUSIONS: We designed a glycopeptide amphiphile that self-assembles into a supramolecular hydrogel that mimic the proteoglycans present in the ECM. Moreover, we were able to pattern grooves in this hydrogel that induced the alignment of cardiomyocytes. The Fmoc-FF-GlcN6S hydrogels are cytocompatibility and maintain the phenotype of the cardiomyocytes, i.e. their contractility was observed by live imaging (markers Trop T and PhL). Fmoc-FF-GlcN6S gels are a promising biomaterial to be used in cardiac tissue regeneration.

ACKNOWLEDGEMENTS: We acknowledge the financial support from the EC(#872648-MEPHOS) and FCT (PTDC/CTM-REF/0022/2020-OncoNeoTreat,PD/BD/135256/2017,COVID/BD/152018/2021 and CEECINST/00077/2018).

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4.He, C.;J Am Chem Soc2020, 142 (40), 17015-17023.

Keywords: Biomaterials, Cardiovascular

PP-050

Tunable glycopeptide-based supramolecular hydrogels drive the differentiation of stem cells into neural lineages

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INTRODUCTION: Cardiac tissue regeneration is still a challenge in tissue engineering and regenerative medicine. A functional recovery of the cardiac tissue requires an adequate contractility of exogenous and/or endogenous cardiomyocytes.¹⁻² Different biomaterials can be used to induce such contractility. Herein, we propose the use of glycopeptide supramolecular gels. Due to the non-covalent nature of the supramolecular interactions (e.g. π - π , CH- π), it is possible to generate dynamic systems³ that respond to different (bio)chemical stimuli. Moreover, these hydrogels can be micropatterned in order to induce the alignment of cardiomyocytes as in cardiac tissue. Glycopeptides copycat the glycoproteins of the ECM and thus, are closer ECM mimic at biofunctional term.⁴

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- 4.He, C.;J Am Chem Soc2020, 142 (40), 17015-17023.

Keywords: Biomaterials, Differentiation

PP-051

Self-Reinforcing Polymers – A New Type of Biomaterials

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INTRODUCTION: High performance polymers often have a narrow processing window, which is a crucial bottle neck in technology, including scaffolds for regenerative medicine. Unfavorable compromises between the processability and the final material properties must be made.[1] The physical interaction between the polymer chains of thermoplasts or thermosets are prerequisite for their high performance, however, also contribute to high solution or melt viscosity and low solubility. Hence, it has been desirable to develop a new class of polymers, which are easily processed and establish their high-performance properties in downstream processes ideally in situ. Self-reinforcing polyurethane ureas (srPUUs) are biocompatible polymers, which transform just by preconditioning them in aqueous media at body temperature and thereby forming polymers with increased strength, extensibility, and toughness.[2] This is achievable by including dynamic hindered urea bonds (HUBs) [3]; In water, these bonds transform to non-hindered urea bonds by a concerted sequence of chemical conversions.

METHODS: We systematically investigated the behavior of srPUUs by variation of components – macrodiol, isocyanate and HUB – and characterized properties before and after preconditioning. Polymers were synthesized by established prepolymer method.[4] Obtained polymers were solution-casted to films; Films were cut in half and either stored dry or in PBS solution. The mechanical properties and microstructure of films were compared by tensile testing and AFM, respectively.

RESULTS: Self-reinforcing was observed for polymers containing HUBs; An increase in tensile strength of polymer films of more than 100% was found. The effect can be tailored by amount and quality of HUBs. AFM indicates a consultation of hard segments during preconditioning, which is basis for self-reinforcing.

DISCUSSION & CONCLUSIONS: Self-reinforcing polymers are an interesting new concept and valuable complement to established polymers used for scaffolds in tissue regeneration. Still this class of polymeric materials needs further research to fully understand the underlying effects, enabling a rational design of tailor-made polymers for specific applications in tissue engineering and regenerative medicine. Currently, this new type of polymers are promising material candidates for electrospun vascular grafts.[4]

ACKNOWLEDGEMENTS: Christian Doppler Research Association, Austrian Federal Ministry for Digital and Economic Affairs, National Foundation for Research, Technology and Development

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Keywords: Polymers - natural / synthetic / responsive, Biomaterials

PP-052

Soft Nanomechanical Mapping of Polyether Ether Ketone (PEEK) via Atomic Force Microscopy

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INTRODUCTION: Polyether ether ketone (PEEK) is a biocompatible, semicrystalline polymer with vast potential as implant material in dentistry and orthopaedics, where it is mainly studied in relation to the addition of nano components to improve bioactivity and mechanical strength. Atomic Force Microscopy-based nanoindentation can be used in force-volume mode (AFM-FV) for mapping the nanomechanical properties of complex biopolymers [1]. However, a lack is found in literature as concerns PEEK indentation under purely elastic indentation regime, where low ($< 1 \mu\text{N}$) forces and small ($\sim 10 \text{ nm}$) penetration depths are potentially able to capture local gradients of the elastic properties relevant to biological processes. The application of AFM-FV under soft nanoindentation regime to medical grade PEEK is the objective of this work.

METHODS: Medical grade PEEK slices ($10 \times 10 \times 6 \text{ mm}$ height, Direct Plastics Ltd., Sheffield, UK) were characterized by an NT-MDT (Moscow, Russia) AFM system equipped with 9 to 30 N/m -stiff cantilevers.

RESULTS: This study characterized the nanoscale mechanical properties of medical grade PEEK by AFM-FV for mapping the maximum penetration depth (h), elasticity index (η) and indentation moduli (E) according to Hertz model at different regions of the surface. The maximum force loads was varied between 100 and 500 nN.

On the scale of $5 \times 5 \mu\text{m}^2$ considered here, predominant elastic ($\eta > 0.5$) response was maintained, and a twofold distribution of E , namely ~ 0.6 and $\sim 1.0 \text{ GPa}$ (low and high) was found. The high moduli resulted distributed across purely elastic ($\eta \sim 1$) and spatially localized islands, typically $< 1 \mu\text{m}$ -large, while the low moduli were mainly spread over larger areas.

DISCUSSION & CONCLUSIONS: The maps of η and E , acquired simultaneously with the topographies, highlight the point-dependent character of the surface mechanics observed in other polymer structures, revealing the presence of localized islands which may be associated to the presence of a double-phase amorphous and crystalline at the free surface, never clearly observed so far.

Our results are interesting as they were obtained by a standard AFM setup, and thus promote AFM-FV as viable tool for characterizing the soft nanoindentation regime of complex biopolymers and to guide optimization of micro and nanostructures for biomedical applications.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

PP-053

Decoding the synergy between PLGA and natural polymers in tissue regeneration

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INTRODUCTION: Tissue engineering opens doors to the possibility of organ and tissue replacement through neo-tissue formation in vitro/vivo, aiming to tackle the dependency of direct transplantations. Natural (fibrin, elastin and collagen) and synthetic (PLGA) polymers have been used to improve the formation of extracellular matrix (ECM) for tissue regeneration. PLGA has shown biodegradability and biocompatibility and is FDA approved for use in tissues. Fibrin, Elastin and Collagen, readily available in the ECM of tissues, ensure tissue regeneration and healing in natural state.

Taking these benefits into consideration, we created and evaluated synthetic/natural polymers seeded with human adipose-derived stem cells to determine an ideal polymer for future clinical use in organ and tissue transplantation.

METHODS: PLGA scaffolds were electrospun and coated with either fibrin, elastin or collagen, or a combination of them, at different ratios.

Degradation, dynamic water contact angle (dWCA), swelling ratio and porosity (using SEM) were assessed

Human Adipose derived stem cells (hADSCs) were seeded on the scaffolds and tested for proliferation and cell viability over time.

In addition to that, scaffolds were placed onto the chorion allantoic membrane (CAM) of chicken embryos to determine their angiogenic capacity.

The differentiation activity and pathways of mesenchymal stem cells, adipogenic, chondrogenic and osteogenic changes were evaluated.

RESULTS: Most of the scaffolds had porosity of $<50\mu\text{m}^2$, that did not impede proliferation, cell migration or angiogenesis. PLGA scaffolds were shown to be more hydrophobic and degraded slower than the other coated scaffolds. However, the angiogenesis and cell proliferation were higher in coated PLGA. Uncoated PLGA and Fibrin coated scaffolds had the highest porosity at 46.85%, 46.81% respectively and showed the highest angiogenesis in the CAM assay.

DISCUSSION & CONCLUSIONS: This study shows that there is no significant difference in cell communication between uncoated PLGA and PLGA coated with collagen and/or fibrin. However, uncoated PLGA scaffolds show a higher metabolic activity and proliferation than coated scaffolds. Moreover, neither pore size bigger than $>50\mu\text{m}^2$ nor natural polymer coating, may be needed for tissue regeneration. These promising findings encourage us to further develop PLGA with or without natural polymers such as collagen and fibrin for future clinical applications in tissue regeneration.

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Keywords: Innovation, Polymers - natural / synthetic / responsive

PP-054

Supercritical Carbon Dioxide Sterilised Decellularised Porcine Pulmonary Heart Valves: Biological and Biomechanical Characterisation

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INTRODUCTION: Decellularised pulmonary valve roots are a promising regenerative solution for reconstruction of the right ventricular outflow tract in young patients. Application of a robust terminal sterilisation process would enhance the sterility assurance level, improve logistics, and expedite clinical translation. Supercritical carbon dioxide (ScCO₂), combined with oxidizers, may provide the ideal sterilisation conditions for decellularised biological scaffolds [1,2]. The aim of this study was to compare the biological and biomechanical properties of decellularised pulmonary valve roots (dPHV), with dPHV exposed to ScCO₂ sterilisation cycles.

METHODS: Low concentration detergent decellularised porcine pulmonary valves (n=6 in each group) were stored in phosphate buffered saline (PBS controls), exposed to ScCO₂ cycles whilst immersed in PBS (ScCO₂-wet), or exposed to ScCO₂ cycles without immersion (ScCO₂-dry). Histological analysis of the samples included Haematoxylin and Eosin, Picrosirius Red-Millers, and Movat's Pentachrome. Presence of collagen type IV and fibronectin was detected by immunohistochemistry. Uniaxial tensile testing was used to determine the dPHV wall and leaflet material biomechanical properties, in the radial and circumferential direction, differential scanning calorimetry (DSC) to determine thermal stability of collagen, and colorimetric assays to quantify collagen and GAG content.

RESULTS: Other than compression of the ScCO₂-dry samples, there were no differences observed within the histoarchitecture and microscopic structures of ScCO₂-wet and ScCO₂-dry groups, or collagen and GAG content, in comparison to the dPHV controls. There was a significant reduction in the thermal stability of the ScCO₂-wet and ScCO₂-dry treated dPHVs, and a significant increase in the ultimate tensile strength of ScCO₂-dry leaflets (radial direction). Significant increases in the elastin phase slope were determined in the circumferential direction of ScCO₂-dry (wall and leaflet) and ScCO₂-wet (wall only), as well as in the axial direction of ScCO₂-dry (wall and leaflet) and ScCO₂-wet (leaflet only).

DISCUSSION & CONCLUSIONS: With further process optimisation, ScCO₂-wet remains a promising sterilisation option for decellularised pulmonary valve roots. The role of the oxidising additives in the detected thermal stability and material property changes requires further investigation, as does the capacity for cellular repopulation.

ACKNOWLEDGEMENTS: We would like to acknowledge the expert advice provided by Novasterilis (USA) in the application of ScCO₂ sterilisation technology.

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Keywords: Decellularised matrices, Cardiovascular

PP-055

Cellular Behaviors of Human Skeletal Muscle Satellite Cells on Tissue Specific Extracellular Matrix Coating and Hydrogels

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INTRODUCTION: Decellularized tissues obtained by removing cellular components from biological tissues is well-known that the effect on the microstructure and constituents differ depending on the tissue source and decellularization method. Previously, we have investigated the decellularization of various tissues using the detergent method, which is a general decellularization method, and the high hydrostatic pressure (HHP) method, which was originally developed. Recently, we demonstrated that the reconstructed decellularized extracellular matrix (dECM) hydrogel, which eliminated the tissue-specific microstructure by enzymatic solubilization of decellularized tissue, exhibits different cellular behavior from collagen gel. However, decellularization of biological tissues not comprising collagen as the major component, such as muscle tissue, has hardly been investigated, and the differences in gel-forming ability and characteristics between decellularized muscle tissue and non-muscle tissue have not yet been demonstrated. In this study, we investigated gel formation of solubilized decellularized muscle tissue and physical properties of the obtained dECM hydrogel. Moreover, we also investigated the effects of dECM coating and dECM hydrogel prepared from decellularized muscle tissue and non-muscle tissue on the adhesion, proliferation and differentiation of skeletal muscle satellite cells (HskMSC).

METHODS: Porcine tongue, psoas major muscle and urinary bladder were used. The detergent (SDC-TX) method and the HHP method were used for decellularization. Decellularization was evaluated by hematoxylin-eosin staining and quantification of residual DNA. The major composition of decellularized tissues was evaluated by collagen and protein quantification. The gel-forming ability was evaluated by dynamic rheological analysis. HskMSCs were seeded on various 2D-dECM coating and 3D-dECM hydrogel and stained with Calcein-AM to evaluate adhesion and proliferation over time. The formed myotubes were stained with anti-skeletal myosin antibody, and the myotube differentiation rate was quantified.

RESULTS: It demonstrated that dECM hydrogels can be prepared from all solubilized solution of decellularized tissues, however the HHP-decellularized muscle tissue tended to form fragile gels compared to the decellularized non-muscle tissue. When HskMSCs were seeded on each dECM-coated substrate to investigate the effects of tissue-specific dECM, it found that the number of myotube formations on the decellularized muscle ECM coating increased compared to other dECM coatings. Little difference was observed due to the difference in decellularization method.

DISCUSSION & CONCLUSIONS: It is considered that this phenomenon is maybe due to the difference the constituents of the tissue source. Taken together, it was suggested that the decellularized muscle tissue contained bioactive substances that act on the myotube formation of HskMSC.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: Decellularised matrices, Hydrogels and injectable systems

PP-056

Parallel multi-structure decellularisation of a porcine three-dimensional right ventricular outflow tract valved xenograft for use in paediatric congenital cardiac surgery

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INTRODUCTION: Tetralogy of Fallot is a cyanotic congenital heart disease affecting 1:3000 live births, requiring right ventricular outflow tract (RVOT) replacement in infancy. Current RVOT substitutes are suboptimal for paediatrics, functionally deteriorating and necessitating surgical reintervention. An optimal prosthetic that mimics the structural and mechanical properties of native tissue is desirable. Due to insufficient homograft availability for neonate and infant patients, xenografts are commonly used in paediatric cardiac surgery. Current xenografts are limited by lack of growth, unacceptable levels of inflammation, susceptibility to calcification, and inadequate remodelling capacity, ultimately resulting in graft rejection. Decellularisation to remove immunogenic antigens such as gal α 1-3Gal β 1-4GlcNAc-R on the animal-derived tissue reduces the risk of graft rejection. Decellularisation conditions are critical: harsh treatment will disrupt extracellular matrix (ECM) components vital for recellularisation; conversely, too mild treatment may lead to incomplete cell removal and graft degeneration. Decellularisation of valved conduits poses a unique challenge as the valve structure differs significantly to that of the artery. The latter is thicker and stronger, requiring harsher detergents which could damage the valve.

This research optimises an in vitro decellularisation protocol for simultaneous removal of porcine cells from the right ventricle (RV), pulmonary artery (PA), and pulmonary valve (PV). Future work aims to recellularise the cell-free scaffold with autologous mesenchymal stem cells, endowing the xenograft with growth capacity and improved biocompatibility compared to existing products.

METHODS: Integrated valved RVOT conduits were harvested from pigs and mounted in a temperature-controlled bioreactor chamber. Decellularisation solutions were circulated through the lumen of the submerged conduit under constant flow, with extended sodium dodecyl sulfate, protease inhibitor, and nuclease washes. Histological assessment of the RV, PA, and PV for nuclei detection, elastin, and collagen content reports on decellularisation success.

RESULTS: Preliminary results demonstrate effective decellularisation of the RV, PA, and PV in both valved conduits decellularised immediately after harvesting, and those decellularised following one freeze-thaw cycle. The ability to freeze RVOTs until needed would facilitate production of an accessible surgical product. Histological data show significant nuclei reduction in each tissue architecture, whilst the gross ECM composition appears unchanged.

DISCUSSION & CONCLUSIONS: Initial data from the concurrent multi-tissue decellularisation methodology reveal promising results. Further experiments are underway to evaluate mechanical properties of the cell-free RVOT, as well as assessment of xeno-antigen removal. Quantification of DNA, soluble collagen, and total protein will also be necessary to deem decellularisation successful.

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Keywords: Cardiovascular, Bioreactors

PP-057

Investigation of microwave-assisted decellularization of bone matrix

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INTRODUCTION: With the increasing demand for bone graft transplantation in the reconstruction of bone defects, various natural biomaterials have been developed as a substitute for autologous bone grafts and allografts. Some natural biomaterials possess the ideal nanostructure to support bone growth. However, when natural biomaterials are introduced in vivo, they will be recognized as exogenous substances and will elicit an immune response. Decellularization is an essential procedure to remove the immunogenic cellular material from bone tissues prior to implantation to reduce the risk of eliciting an immune response. The length and complexity of a decellularization protocol vary depending on the structure and properties of the material. In particular, the decellularization of hard and dense materials such as animal bone tissues is a long and tedious process.

METHODS: This study describes a novel bone decellularization technique utilizing microwave energy to accelerate the decellularization process and achieve effective decellularization. Herein, we compared the conventional method, which is a combination of freeze-thawing, osmotic shock, enzymatic digestion and vacuum-assisted decellularization with the novel microwave-accelerated decellularization method.

RESULTS: Unlike the conventional method which required two weeks, the microwave-accelerated method can be completed within a significantly shorter span of one week. DNA quantification results showed a significant reduction in DNA content despite the much shorter decellularization time.

DISCUSSION & CONCLUSIONS: This novel application of microwave energy to accelerate the conventional bone decellularization procedures will facilitate the pre-treatment of natural biomaterials for bone tissue reconstruction.

Keywords: Decellularised matrices, Biomaterials

PP-058

Bone matrix derived bone-tendon enthesis for repair of irreparable rotator cuff tears

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INTRODUCTION: The junction between tendon and bone is a progressively evolved “multi-tissue” structure: tendon-fibrocartilage - mineralized fibrocartilage - bone. These tissues have progressively increasing Young’s moduli, which distribute the forces generated in the muscles onto the bone. In this study, we developed a novel bone matrix processing method and developed a naturally derived bone-tendon enthesis.

METHODS: Optimisation samples of 15x10x5mm³, trialled as separate cancellous and cortical bone samples, were cut from porcine femoral condyles and shafts, respectively. Samples underwent 1-week progressive stepwise decellularisation consisting of freeze-thaw, ionic shock and enzymatic digestion, with or without the addition of sonication and negative pressure vacuum, comparing controls in phosphate buffered saline only. Samples were weighed before and after decellularisation, then processed histologically to review cellular staining. For partial demineralisation, samples were bathed in hydrochloric or ethylenediaminetetraacetic acid, with mineral protection afforded by embedding half the sample in paraffin wax or covering with a nitrocellulose polymer solution.

RESULTS: An initial device design of rectangular cuboidal shape with dimension ratios of 3:2:1 was established. The addition of sonication and negative pressure vacuum improved the effectiveness of the decellularisation process; cancellous samples decreased in wet mass post-decellularisation by 45.3% ($p<0.001$), compared to 18.1% ($p<0.05$) without sonication and vacuum, with a reduction in cellular content on haematoxylin and eosin staining. Cortical and control samples did not show a significant decrease in wet mass. Acid bathing and protection methods provided partial demineralisation protection.

DISCUSSION & CONCLUSIONS: Initial optimisation trials show promise in the design of a soft-hard tissue enthesis scaffold as an immune compatible xenograft to provide new therapeutic options for irreparable rotator cuff tears. The addition of sonication and vacuum negative pressure will be essential for timely decellularisation, and further studies of biological, mechanical and structural characterisation are underway to assess device validity, before in vivo animal model trials and potential clinical translation.

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Keywords: Decellularised matrices, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-059

An induced pluripotent stem cell-based model to study the role of extracellular matrix in myocardial fibrosis

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INTRODUCTION: Cardiac fibrosis is the consequence of chronic insults to the myocardium, characterized by the abnormal accumulation of extracellular matrix (ECM). Differentiation of cardiac fibroblasts (cFbs) into myofibroblasts drives the pathological ECM remodelling, a process highlighted by biochemical and structural changes which compromises cardiomyocytes (CMs) contractile activity and eventually leads to heart failure [1].

METHODS: Here, we adopted bioengineering tools and induced pluripotent stem cells (iPSCs) to investigate how fibrotic ECM affects the structural and functional properties of CMs.

RESULTS: Thus, we derived cFbs from induced pluripotent stem cells (iPSCs-cFbs), and optimized a protocol to induce their differentiation into myofibroblasts based on fine-tuning TGF-beta signalling. Next, we obtained fibrotic ECM (dECM) by implementing a decellularization procedure of the activated iPSCs-cFbs and analyzed the pathological changes occurring during the deposition of diseased cardiac ECM. Then, we developed iPSCs-CMs and cultured them either on healthy or fibrotic dECM. Morphological and functional analysis were implemented to study how the biomechanical properties of pathological ECM affect CMs physiology and function.

We further validated our model using cardiomyocytes differentiated from iPSCs derived from Duchenne muscular dystrophy (DMD) cardiopathic patients [2].

Finally, we established a 3D in vitro culture system, which entails the co-culture of isogenic iPSCs-CMs and -cFbs that better reproduces the cellular complexity and functionality of the human heart and represents a powerful tool for personalized medicine applications [3].

DISCUSSION & CONCLUSIONS: By capitalizing on this approach, we might be able to recapitulate the accumulation of fibrotic tissue during heart disease and investigate the contribution of pathological ECM to the progression of heart failure and many other cardiac pathologies.

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Keywords: Decellularised matrices, Induced pluripotent stem cells

PP-060

Challenges and pitfalls for determination of DNA content in decellularized skeletal muscle tissue

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INTRODUCTION: Biomaterials based on decellularized tissue are increasingly being used within the field of tissue engineering and regenerative medicine. The wide variety of decellularization protocols to obtain acellular matrices urges the need for suitable evaluation methods. In this context, determination of residual DNA content is of particular importance to avoid an adverse immune response upon transplantation. However, different qualitative and quantitative methods are being used and it remains unclear which method is most appropriate.

METHODS: In this study, our goal was to determine the DNA content of decellularized skeletal muscle tissue obtained with four different decellularization protocols. Samples were first lyophilized to determine dry weight. For quantitative DNA assessment, samples were either processed with the DNeasy Blood and Tissue Kit of Qiagen or incubated overnight with papain and further quantified with the Qubit dsDNA broad range assay kit. In addition, gel electrophoresis was performed to visualize any residual DNA fragments and histological Feulgen and DAPI staining was used for qualitative analysis.

RESULTS: For three out of four decellularization protocols, both methods for quantitative evaluation of residual DNA reflect the decellularization status of the samples as seen with histological staining. For one decellularization protocol, however, the DNA content relative to tissue dry weight is remarkably higher than in native tissue and not consistent with the histological staining which indicates some DNA removal. We hypothesize this is due to the major impact of this decellularization protocol on the extracellular matrix. Interestingly, for samples which are not or incompletely decellularized, DNA quantification after silica-based extraction leads to higher absolute values of DNA content compared to measurement immediately after papain digestion. On the other hand, for efficiently decellularized samples, lower absolute values are observed when using the DNeasy Blood and Tissue Kit. Gel electrophoresis demonstrates that mostly smaller DNA fragments, which are still present after enzymatic digestion, are lost after silica-based extraction.

DISCUSSION & CONCLUSIONS: Quantitative expression of DNA content relative to tissue dry weight warrants some caution, as this ratio is also influenced by impact of the decellularization on the extracellular matrix. Furthermore, direct measurement of DNA content after digestion with papain is preferred over silica-based DNA extraction. A combined approach including both quantitative and qualitative evaluation methods is necessary to fully understand the impact of decellularization on the DNA content of a tissue.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc),

PP-061

Cisplatin Loaded Graphene Oxide Nanoplatforms to Enhance Drug Delivery in Anticancer Therapy

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INTRODUCTION: Chemotherapy is largely used to treat cancer and it is based on the use of molecules targeting the high cancer cell proliferation metabolism by binding nuclear DNA upon overpassing the cell membrane, causing its damage and the arrest of the cancer cell cycle at G2/M transition phase, leading to apoptosis.(1-3) Despite Pt chemotherapeutics are the most potent used anticancer drugs success their side effects (high degradation before entering the cells, the off-target organs toxicity, and cell resistance) remain great drawbacks.(4,5) An interesting strategy to overcome such limitations is the development of nanocarriers that could enhance cellular accumulation in target cells in addition to decreasing associated drug toxicity in normal cells.

METHODS: In this study we compared clinically used Platinum (Pt-free), graphene oxide nanoplatforms bonded to Pt by polyethylene glycol (Go-PEG-Pt), and PEGylated graphene oxide (Go-PEG) on seven cancer cell lines (MG63, U2-OS and SAOS-2 osteosarcoma cell lines; MDA-MB-231 and MDA-MB-468 adenocarcinoma cell lines; U87 and U118 glioblastoma cell lines) at three different Pt concentrations (15µM, 30µM, and 60µM), investigating their in vitro effect on cellular uptake, viability, morphology and migration.

RESULTS: A preliminary study showed that GO-PEG was not toxic for cells at any concentration tested compared to cells only. The cell viability was significantly reduced in MDA-MB-468, U118 and all osteosarcoma cell lines at 30µM for GO-PEG-Pt group compared to Pt-free. These results were highly related to the cellular uptake of GO-PEG-Pt which is significantly higher compared to Pt-free after 24h. Also cell morphology was compromised in a dose dependent manner in the presence of GO-PEG-Pt respect to Pt-free. In addition, GO-PEG-Pt mostly affected the cell migration compared to Pt-free, in particular, MDA-MB-231, MG63 and U2-OS showed a migration reduction of 60%, and this could be a great advantage in reducing the metastasis process.

DISCUSSION & CONCLUSIONS: In this work we demonstrated that GO-PEG-Pt platforms are a promising anticancer delivery system by providing numerous advantages such as minimizing toxicity,

enhancing the cellular uptake, and consequently we could reduce the side effects because a lower amount of Pt is necessary.

ACKNOWLEDGEMENTS:

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Keywords: Drug delivery, Nanomaterials (inc graphene)

PP-062

Graphene Oxide affects the Epithelial Mesenchymal Transition and immunomodulatory properties of Amniotic Epithelial Stem Cells

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INTRODUCTION: The application of immune regenerative strategies to deal with unsolved pathologies, such as tendinopathies, is getting attention in the field of tissue engineering exploiting the innate immunomodulatory potential of stem cells [1]. In this context, Graphene-Oxide (GO) showed to affect stem cells behaviour and to modulate their immune response [2]. Starting from this premise, Amniotic Epithelial Cells (AECs), a stem cell source known for teno-differentiation and immunomodulatory properties both *in vitro* and *in vivo* [3], are used to study the GO effect on AEC phenotype and immune paracrine activity in the presence or absence of pro-inflammatory inductive stimulus (+/- LPS)

METHODS: AECs isolated from the amniotic membrane obtained from ovine fetuses [4] were seeded under standard cultural condition (AEC) or on GO functionalized cover slides (GO). After confluence, AECs were stimulated 1h with 1 µg/ml LPS [4] (AEC+LPS, GO+LPS), followed by 24h of culture in serum-free-media before cells and conditioned media (CMs) collections. GO effect was studied by analysing the Epithelial-Mesenchymal-Transition (EMT) through real time-PCR, immunofluorescence for Cytokeratin 8 (CYTO8), epithelial, and Vimentin (VIM), mesenchymal markers as well as for the activation of TGFβ/SMAD signalling pathway analysed through Western Blot and TGFβ/SMAD reporter kit. Moreover, the paracrine activity of cell derived- CMs was assessed by combining molecular (Inflammation Array Membrane) and functional (immune cells 'assays) analyses.

RESULTS: The results showed that GO promotes the AEC EMT independently of LPS stimulation by inducing morphological and molecular mesenchymal profiles as indicated by CYTO8- and VIM+. Moreover, EMT induced by GO was TGFβ/SMAD-dependent as confirmed by the upregulation of pSMAD in the GO primed groups and EMT prevention in after SB-505124 treatment. Additionally, membrane array data suggested that GO was able to influence the AEC cytokine release profile, reducing the anti-inflammatory release of chemokines such as CXCL9, TIMP2, IL10, IL11 and IL13 after LPS treatment and impact on the activation of monocytes.

DISCUSSION & CONCLUSIONS: These results demonstrate that GO accelerates the EMT process in AEC, by activating TGFβ/SMAD signaling pathway. Moreover, GO modulates stem cells immune paracrine activity influencing the release of immune modulatory molecules. Further experiments are being performed to confirm the CMs immunomodulatory properties on different source of immune cells (PBMCs, monocytes, macrophages M1 and M2).

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Keywords: Stem cells - general, Immunity / immunomodulation / macrophage

PP-063

Magnetoliposomes for intraarticular delivery of ascorbic acid and dexamethasone

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INTRODUCTION: Osteoarthritis (OA) is a degenerative disease that currently does not benefit a disease modifying therapy. Limitations for the development of innovative therapeutics are mainly related to the complexity of the underlying pathogenic mechanisms as well as the unmet need for relevant disease models. Magnetic nanoparticles (MNPs) have been proposed as an efficient modality for the delivery of bioactive molecules within OA joints limiting the side effects encountered with systemic delivery. In this work, proprietary Fe₃O₄ MNPs formulated as oil-in-water (O/W) magnetoliposomes (MLs) containing ascorbic acid and dexamethasone were tested for size, stability, magnetic properties and biocompatibility as well as anti-senescent and proliferative potential in vitro.

METHODS: MLs were formulated using Fe₃O₄ nanoparticles, oleic acid or tocoferol, dexametasone and ascorbic acid by a mixed low-energy (spontaneous emulsification) and high-energy (ultrasonication) method. Dynamic light scattering (DLS), XRD, TEM, and FTIR were performed for size and content characterization while magnetization was assessed by vibrating sample magnetometry (VSM). Tests of cellular viability, proliferation, and senescence were performed on human dermal fibroblasts and chondrocytes using MTT. LIVE/DEAD assay and fluorescent beta galactosidase activity.

RESULTS: The magnetoliposomes were found to be nanosized in the range of 10-300 nm, with high stability in solution without phase separation, cream formation or flocculation. Also, they remained very stable in solution for 3 months with very favorable magnetic reactivity. An excellent cell viability and proliferation was observed up to 21 days in culture while culture-induced senescence was decreased compared to controls in both chondrocyte and fibroblasts.

DISCUSSION & CONCLUSIONS: We successfully developed MLs that carry bioactive molecules, such as ascorbic acid and dexamethasone, that provide long-term stability, magnetic responsiveness and improvement of chondrocyte proliferation and decrease of senescence in vitro.

Future studies are warranted to assess anti-inflammatory and regenerative properties both in vitro and in vivo for the establishment of a possible intraarticular delivery therapeutic method for OA

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Keywords: Biomaterials, Drug delivery

PP-064

Fabrication of Anodized Zirconia Dental Implants towards Enhanced Bioactivity

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INTRODUCTION: Attributed to their excellent physical, mechanical and biological properties, Zr-based implants are rapidly gaining popularity [1]. While Zr-based dental implants have shown promising outcomes, long-term success may be challenged, especially in compromised conditions. To cater to such conditions, conventional Zr implants have been modified via physical, chemical and biological means to augment cellular functions to achieve enhanced osseointegration, soft-tissue integration and local therapy [2-3]. This research demonstrates the optimized fabrication of ZrO₂ nanostructures (ZON) on clinical implant-relevant micro-machined and rough surfaces. Our approach bypasses the conventional implant polishing, thereby preserving the underlying micro-roughness that is crucial for osseointegration.

The project aims to:

1. Fabricate ZrO₂ nanostructures (ZON) onto Zr-based implants via anodization.
2. Understand the mechanism of formation and perform in-depth characterization.
3. Investigate the bioactivity of ZON using osteoblasts *in vitro*.

METHODS: ZONs were fabricated on Zr flat foil and Zr wire (model for abutment) using electrochemical anodization at various voltages/times. Both Rough- (as received) and Micro- (micro-machined) Zr were used as substrates. Characterization was performed using SEM, AFM, XPS, XRD and water contact angles. Further, primary human osteoblasts were cultured on the surface of ZONs *in vitro*, followed by evaluation of proliferation and adhesion.

RESULTS: Randomized ZONs were fabricated on Rough-Zr, while aligned ZONs were formed on Micro-Zr. Further, evidence of the effect of anodization voltage was evident, with the preservation of underlying micro-architecture to form dual micro-nano structures. ZONs promoted the proliferation of osteoblasts and the aligned ZONs mechanically stimulated cells to align in a parallel direction.

DISCUSSION & CONCLUSIONS: Dependent on substrate topography, voltage and time, aligned or random zirconia nanostructures can be fabricated. Further, nano-engineered implants enhanced the function of osteoblasts and enabled their alignment parallel to the nanostructures. Controlled nanostructures fabricated on complex implant topography are a promising strategy for translating nano-engineered Zr into the current implant market.

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Keywords: Biomaterials, Nanomaterials (inc graphene)

PP-065

Dopamine functionalized PANi/SF/PCL based electroactive bilayer fiber substrate for enhanced Osteogenesis

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INTRODUCTION: Electroactive polymer such as PANi (Polyaniline) are promising platform for manipulating electrical stimulus to MSCs for engendering the differentiation for enhanced bone regeneration. We report fabrication of bilayer nanofiber substrate through electrospinning technique where the base layer of scaffold was constructed from SF(Silk fibroin)//PCL(Polycaprolactone) using emulsion system followed by top layer of PANi/SF/PCL using single solvent system. A high SF content loaded in both the layers balances the cytotoxic effect of synthetic polymer and demonstrated inducing opportunity for bone tissue regeneration.

METHODS: The bilayer electroactive electrospun fiber substrate was further functionalized using dopamine and were characterized by FESEM, FTIR and XRD.

RESULTS: The mechanical properties of the functionalized bilayer showed young's modulus upto 45 MPa and high surface roughness to support the MSCs proliferation. The degradability, wettability, protein adsorption and swelling study were also performed. The functionalized bilayer scaffold promoted MSCs cell proliferation, viability and differentiation.

DISCUSSION & CONCLUSIONS: Without electrical stimulus the induction culture for 14 and 21 days differentiation study showed promising osteogenesis gene expression by mRNA quantification of OCN, OPN, ONC and ALP detected by RT-PCR. The results confirms the enhanced osteogenic differentiation on dopamine functionalized bilayer electroactive scaffold.

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Keywords: Differentiation, Electroactive materials

PP-066

Additive Manufacturing of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-Based Composites for Bone Regeneration

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INTRODUCTION: Polyhydroxyalkanoates are biopolyesters synthesized by various microorganisms. Thanks to their biodegradability, mechanical properties and processing versatility, they have become unique candidates for research and industrial application [1]. Additive Manufacturing (AM) uses advanced technologies to fabricate parts by joining materials layer-by-layer. Their ability to fabricate 3D objects directly from a digital representation makes them excellent alternatives compared to traditional manufacturing [2]. In this contribution poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) processing in combination with osteoinductive inorganic fillers was investigated by employing computer-aided wet-spinning (CAWS), a solution/suspension-extrusion AM technique. The effect of PHBV blending with poly(D,L-lactide-co-glycolide) (PLGA), an amorphous poly(α -hydroxyacid), on processing and the fabricated prototypes properties was also evaluated to develop optimized scaffolds for bone regeneration.

METHODS: PHBV, PLGA, and hydroxyapatite (HA) particles were blended as a chloroform suspension and processed by CAWS through extrusion into an ethanol coagulation bath. The fabricated prototypes were characterized by scanning electron microscopy (SEM), contact angle (CA) measurements, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), compression tests, and preliminary in vitro cell culture tests.

RESULTS: 3D porous prototypes were fabricated by CAWS starting from PHBV/HA and PHBV/PLGA/HA suspensions at different weight ratio. SEM analysis on the obtained structures showed better aligned and smoother fibers for PHBV/PLGA/HA samples, as well as a more defined porosity along z axis and the presence of two distinct polymeric phases resulting, for particular blend compositions, in a microfibrillar morphology of the dispersed phase. CA measurements showed an increase of scaffold wettability by increasing PLGA content in the blend. Thermal analysis showed an increase in the degradation temperature and a decrease of crystallinity after blend formation, while compression tests revealed a comparable modulus for the fabricated prototypes. Scaffolds also sustained in vitro proliferation and differentiation of murine preosteoblasts.

DISCUSSION & CONCLUSIONS: The better processability of PHBV/PLGA/HA suspensions compared with PHBV/HA ones was probably due to an increase in viscosity. This aspect can be exploited to obtain a more precise and reproducible fiber deposition and to control scaffold morphological properties like pores dimension and surface roughness, as well as the intrinsic wettability and crystallinity of the resulting material, without affecting sample's mechanical properties.

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Keywords: Composite materials, Additive manufacturing

PP-067

A hierarchical multifunctional composite scaffold for bone applications: metal additive manufacturing meets tissue engineering

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INTRODUCTION: Despite the tremendous technological advances that have led metal additive manufacturing (AM) to be considered among the most promising approaches to design next-generation prosthetic devices, only a few biomedical applications are available on the market. The lack of knowledge about their structural integrity and the absence of AM standards are still major concerns for an effective scale-up to the industrial field. Further limitations can also arise when dealing with prostheses' biological properties. As a matter of fact, the morphological accuracy of sub-unit elements along with the printing resolution is a severe constraint in the design workspace of lattices, hindering the possibility of manufacturing structures optimized for proper osteointegration.

METHODS: To overcome these issues, the authors developed a new hybrid multifunctional composite scaffold, consisting of an AM Ti6Al4V lattice structure infilled with a silk fibroin/gelatin foam. The composite was realized by combining laser powder bed fusion (L-PBF) of simple cubic lattice structures with gas foaming techniques of the protein blends. Additionally, to further assist the polymer adhesion on metal surfaces, the application of an electric field during foaming, viz electrowetting, has been proposed.

Four different conditions were fabricated, namely SF (silk fibroin), SF_EW (silk fibroin electrowetted), SFG (silk fibroin/gelatin), and SFG_EW (silk fibroin/gelatin electrowetted). Characterizations of the main foam morphological features and adhesive behavior at the metal-polymer interface were performed along with biological in-vitro tests.

RESULTS: The scaffold morphological characterization revealed comparable pore dimensions, but different morphological features, ascribable to the presence or absence of the electric field and to the gelatin addition in the blend solution. The SF_EW composites showed nanofibril structures of silk fibroin, typical of the protein adhesion on metal surfaces, while SFG and SFG_EW specimens appeared to cover the outermost unmelted titanium particles more intimately. The pull-out tests reported the higher adhesion of SFG_EW composites in dry and hydrated conditions, while the biological in-vitro tests confirmed a large proliferative capacity of bone marrow-derived human mesenchymal stem cells.

DISCUSSION & CONCLUSIONS: The SFG_EW reported promising results as filler of titanium implants, due to an enhanced and more intimate protein adhesion on the titanium surfaces, probably ascribable to the binding role of gelatin and a spatial reorganization of silk fibroin–gelatin complexes under the electric field. Furthermore, the preliminary biological results were able to demonstrate the suitability of the proposed multifunctional composite hybrid system as a potential candidate for novel bone implants with improved osteointegration.

Keywords: Additive manufacturing, Biomaterials

PP-068

Novel Piezoelectric and Osteoinductive Electrospun Nanofibers for Bone Tissue Engineering

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INTRODUCTION: The development of an adequate therapy for non-union bone fractures remains largely elusive, with current clinical strategies only achieving suboptimal outcomes. Bone tissue engineering (BTE) strategies focusing on the fabrication of scaffolds to replace damaged tissue and support the regeneration process have been gaining interest. While the piezoelectric properties of bone have been extensively studied, they keep being overlooked in the design of bone scaffolds. The aim of this work was to develop novel hydroxyapatite(HAp)-containing osteoinductive and piezoelectric poly(vinylidene fluoride-co-tetrafluoroethylene)(PVDF-TrFE) nanofibers capable of mimicking both the fibrous nature/inorganic phase of bone extracellular matrix (ECM), and the native piezoelectric properties of bone.

METHODS: PVDF-TrFE/HAp nanofibers with different HAp concentrations (1/3/5/10 wt%) were produced via electrospinning. Fiber morphology was evaluated with SEM and AFM, while the chemical properties of the fibrous scaffolds were analyzed with FTIR and XRD. The effect of scaffold composition on the overall piezoelectric/piezoresistive, hydrophobicity and mechanical properties of the nanofibers was assessed. The bioactivity of the fibers was simulated through an in vitro mineralization assay. Mesenchymal stromal/stem cells (MSCs) were seeded onto the fibrous scaffolds and differentiated into osteoblasts for 21 days. Osteogenic differentiation was evaluated via ALP activity, calcium deposition, gene expression, and immunofluorescence.

RESULTS: PVDF-TrFE/HAp nanofibers with a homogenous HAp nanoparticle distribution and average diameters (231-432nm) within the range of the collagen fibrils found in the native bone ECM were successfully developed. An increase in polar β -phase content of the fibers, associated with augmented piezoelectricity, was observed with the increase in HAp concentration. HAp incorporation also improved scaffold wettability while retaining the mechanical properties of the electrospun fibers. In vitro mineralization was significantly enhanced in the HAp-containing scaffolds. MSC growth was more expressive for the PVDF-TrFE/HAp nanofibers and an augmented ALP activity, cell-derived calcium deposition and bone-related gene expression were observed with the addition of HAp.

DISCUSSION & CONCLUSIONS: Overall, through the addition of HAp to the PVDF-TrFE nanofibers important shifts in the chemical structure of the piezoelectric polymer occurred, being responsible not only for improving the piezoelectricity of the scaffolds but also enhancing the surface properties of the fibers, strengthening their ability to support the mineralization process and cell proliferation. The biological cues provided by the HAp nanoparticles appeared to improve the osteogenic potential of the scaffolds. Our results highlight the potential of the PVDF-TrFE/HAp nanofibers for BTE applications.

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REFERENCES:

Keywords: Fibre technology, Electroactive materials

PP-069

PLGA-carriers for Growth Factor Sustained Delivery within a bioplotted 3D collagen matrix promoted in vitro chondrogenesis of hBM-MSCs

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INTRODUCTION: Articular cartilage lesions are challenging to manage in clinical settings, cannot spontaneously heal [1] and tissue engineering could represent a valid treatment option [2] [3]. In this study, we described a bioengineered three-dimensional (3D) scaffold for induction and maintenance of chondrogenic phenotype assembled with human bone marrow-derived mesenchymal stem cells (hBM-MSCs) in a type I collagen hydrogel functionalized with microcarriers for a controlled release of transforming growth factor $\beta 1$ (TGF- $\beta 1$) as chondrogenic inductor.

METHODS: hTGF $\beta 1$ loaded PLGA carriers (mean size $1 \pm 0.4 \mu\text{m}$) were obtained from double emulsion processed with dense carbon dioxide [4]. Particles were analyzed with respect to their size, morphology, loading and release profiles. hBM-MSCs were positive for CD90, CD105, CD73 and negative for CD14, CD34, CD45, HLA-DR expression (Beckman Coulter, Brea, CA, USA). The 3D bioplotter was Rokit INVIVO 3D printer (Rokit healthcare, Seoul, Korea). Gene expression data by qRT-PCR and histological and quantitative-immunofluorescence (qIF) analysis assays provided indications of cells' behavior along a dynamic or static culture by perfusion.

RESULTS: The tixotropic properties of the collagen hydrogel loaded with carrier assured good cells viability during gel extrusion and PLGA carriers did not interfere with cell viability and crosslinking. Excellent cell vitality was achieved operating with a nozzle diameter of 0.2 mm and operating with a velocity of 5 mm/sec. Cells were cultured under static and dynamic conditions using a perfusion bioreactor, and chondrogenic commitment was studied showing that chondrogenesis was induced (COL2A1, > 5-fold), only under perfusion conditions (see Figure 1). The observed behavior was probably due to the fact that the range of 9-6 ng/day of hTGF $\beta 1$ that is expected to be released within the scaffold in the first 8 days, followed by the mean of 3 ng/day was achieved only under perfusion conditions.

DISCUSSION & CONCLUSIONS: The results suggested that carriers can be successfully bioplotted into a 3D structure acting as a micro-environmental regulators to promote chondrogenic commitment of hosted hBM-MSCs. The versatility of the system enables future design for sustained release of multiple growth factors and/or immunomodulatory signals to explore new solutions for 3D in-vitro cartilage models.

ACKNOWLEDGEMENTS: Acknowledgements to all the Translational and Nanomedicine Lab Research team who support the work.

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Keywords: Biofabrication, 3D printing and bioprinting

PP-070

Quantitative Assessment of the Comparative NP-Uptake Efficiency in a range of biological fluids

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INTRODUCTION: Nanoparticles potentially provide a powerful tool for specific treatments of diseases, acting as a drug delivery transport. However, a deep understanding and control of how nanoparticles interact with biological systems is a key driver to assure the safe implementation of nanomedicine[1, 2]. The overall idea of this project was to provide new leads in the development of such a new field, finding tools for various biomedical applications. What the biological cell actually "sees" when interacting with a nanoparticle will influence the mechanism of internalization. Thus, whether the cell is presented with the bare particle or the particle dispersed in a biological medium, thus covered by a protein corona, results in different uptake behaviour. [3, 4]

METHODS: For this project, multiple cell lines were used and the ultimate goal was to control and quantify uptake of a series of negatively charged carboxylate modified polystyrene of different size, (20nm, 40nm, 100nm, 200nm, 500nm, 1µm) understanding the endocytic pathways required for NPs internalization and their final sub-cellular destination, when NPs were dispersed in different biological fluids (no serum, Foetal Bovine Serum, Human Albumin, Proprietary Blood Fraction)

RESULTS: We found that internalization of nanoparticles is highly size dependent for all cell lines studied, with the different cell types showing very different uptake efficiencies for same materials. Moreover, our studies showed that, in a physiological relevant environment, highly complex protein coronas are established and modulate biological effects at the nanoparticle-cell interface in an innovative way, which has important implications for nanomedicine.

DISCUSSION & CONCLUSIONS: Moreover, our studies showed that, in a physiological relevant environment, highly complex protein coronas are established and modulate biological effects at the nanoparticle-cell interface in an innovative way, which has important implications for nanomedicine.

ACKNOWLEDGEMENTS:

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Keywords: Drug delivery, Nanomaterials (inc graphene)

PP-071

Utilisation of Design of Experiment to Optimise Hydrogel Composition for use in Tissue Engineering

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INTRODUCTION: Challenges arise when developing a bio-ink for 3D bioprinting that allows for precise printability, biocompatibility, ideal mechanical properties, and batch-to-batch consistency. Here, a methodology was designed to optimise a hydrogel-based bio-ink utilising the 'Design of Experiment' (DOE) statistical tool and rheological behaviour of each composition.

METHODS: The 2-level factorial DOE (Minitab®) generated 33 samples of Gelatin (1-5%), Hyaluronic acid (0.5-2%), Dextran-40 (0.5-2%) dissolved in cell-culture media. As our control, Cellink Skin was characterised to establish a target viscosity. The samples from the DOE were subject to an Isothermal Viscosity Test using the MCR-92 Rheometer, that measured viscosity at a constant temperature of 37°C at a high shear rate of 80 s⁻¹.

Using the Cellink BIO-X printer, G-codes were generated to optimise print speed and print pressure. The G-code was programmed to print 20mm lines from 0-50KPa at a constant speed of 5, 10, 15mm/s. Viability of cells post printing were observed using live/dead stains.

RESULTS: The DOE identified that Hyaluronic acid is the dominant influencer on viscosity, in both bio-ink components non-cell laden and cell laden. Hyaluronic Acid increased the viscosity, while Dextran-40 decreased viscosity of the bio-ink as an individual component and combined with Gelatin. The final concentrations of each component utilising the DOE based on the target viscosity of 1271.790mPa/s established the bio-ink composition of 5% Gelatin, 2% Hyaluronic Acid and 0.5% Dextran-40 in both non-cell laden and cell laden samples. The bio-ink was optimised to print at an appropriate print speed and pressure producing cell viability above 70% post printing.

DISCUSSION & CONCLUSIONS: Applying the use of a DOE to this methodology removes the time-consuming methods of trial and error, and to accurately design a bio-ink optimised to a particular target. The use of Gelatin, Hyaluronic Acid and Dextran-40 provides versatility to this bio-ink, while naturally replicating the cellular microenvironment for soft tissue engineering. Using a commercially available Bioink (Cellink Skin) as a target viscosity baseline for this bio-ink enabled assumption to be adopted to predict printing parameters which can be applied during the printing process.

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REFERENCES:

Keywords: 3D printing and bioprinting, Polymers - natural / synthetic / responsive

PP-072

Modelling a dynamic printability window: a different approach to printability assessment studies

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INTRODUCTION: Bioprinting has gained a leading role in the field of bioengineering. Among the available techniques, extrusion-based bioprinting (EBB) is the most widely used, thanks to its affordability and simplicity. The major challenge of this field lays on engineering inks able to fulfill both the processing and the application requirements. Given the novelty of the field and the lack of standardized procedures, the formulation and printing optimization of new inks is still based on lengthy trial-and-error procedures. A consistent research effort has been put into trying to overcome this issue by attempting to predict suitable printing parameters from the ink rheology, however, this matter still requires extensive research. Hereby, we propose a novel model presenting the ideal procedures to identify a “dynamic printability window”.

METHODS: Different compositions of a polysaccharide blend were studied to build two empirical models predicting:

- Rheology of the inks from composition
- Printing accuracy as a function of composition and extruding pressure

The printability window was identified as an area of the printability model by applying constraints on the mass flow and on the spreading ratio. The same area was applied to the rheological model, identifying a range of printable compositions for any given extrusion pressure. The found window was applied to methacrylated versions of the blends to move to multilayered structures by quick layer-by-layer crosslinking. Moreover, a desirability function was built upon the models and used to identify the optimal printing composition and pressure and to predict the outcomes of the printing process.

RESULTS: The model predictability was verified and found suitable to identify the printing quality of chemically modified versions of the blends, allowing the application of the printability window built on planar structures to the manufacturing of multi-layered ones. The desirability function built upon the models proved to be a useful tool to optimize the composition of the ink and the extrusion

pressure.

DISCUSSION & CONCLUSIONS: The models developed are easy to implement, based on cheap measurements and on automatized procedures. The dynamic printability window proved to be a versatile tool, adaptable to chemically modified variations of the studied inks and suitable to build desirability functions for optimization. Future plans cover including new variables in the models to increase precision, and to simulate the inclusion of cells in the inks.

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Keywords: 3D printing and bioprinting, Additive manufacturing

PP-073

Development of an advanced bio-fabrication platform for cartilaginous tissue regeneration

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INTRODUCTION: Over 1.5 million people across the USA and Europe suffer from meniscal injuries every year with the majority progressing to early osteoarthritis. Current surgical therapies provide minimal tissue regeneration and so have poor long-term success rates. Therefore, there is a distinct clinical need for tissue engineered approaches. This project aims to develop an advanced biofabrication pipeline, incorporating microvalve bioprinting and melt-electrowriting (MEW) techniques, to engineer zonally-specific meniscal tissue analogues containing cellular, biochemical, and mechanical gradients that mimic those seen in native tissue.

METHODS: MEW process parameters of extrusion pressure (0.1MPa to 0.5MPa) or collector speed (5-60mm/s) were optimised to enable layer-upon-layer deposition of PCL microfibres with tuneable fibre size. Consecutive layers of straight fibres orientated at 90° to one another were deposited to create microchambers of PCL that function as reinforcement for a cell-laden soft hydrogel matrix. The effect of both fibre size and microchamber size on the stiffness of the PCL constructs was measured through unconfined compression tests. Microvalve jetting of 1.2% alginate into MEW microchambers was developed to maximise resolution and cell viability, and to enable droplet patterning into PCL microchambers. Finally, viability of encapsulated human Y201 MSCs was assessed using a LIVE/DEAD assay.

RESULTS: Straight PCL fibres with diameters analogous to collagen fibre bundles within native cartilaginous tissues (3-20µm) were created. The minimum fibre spacing of PCL fibres enabled creation of microchambers of 100µm wall length. Increasing fibre diameter and decreasing fibre spacing of 1 mm thick scaffolds led to an increase in compressive stiffness approaching physiologically relevant values (~150kPa). The minimum droplet diameter of microvalve jetted 1.2% alginate was found to be ~ 600µm, enabling creation of checkerboard-like patterns of individual alginate droplets in alternating 1000µm PCL microchambers. High MSC viability was observed (>90%), comparable to the manual seeding of such gels.

DISCUSSION & CONCLUSIONS: A biofabrication platform integrating MEW and microvalve bioprinting has been developed with the possibility to tailor fibre sizes, pore architecture, mechanical properties, and cellular gradients. Stiffnesses of the PCL reinforcement come close to native cartilaginous tissue. The ability to pattern bionks into MEW microchambers with chamber-to-chamber resolution has been shown to be possible with high cell viability, thus enabling further application of the developed fibre-reinforced scaffolds in engineering mechanically robust, cartilaginous tissue analogues for tissue regeneration and preclinical disease modelling.

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Keywords: 3D printing and bioprinting, Cartilage / joint and arthritic conditions

PP-074

Development and characterization of 3D bioprinted intervertebral disc model

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INTRODUCTION: The Intervertebral disc (IVD), a fibro-cartilaginous structure, contains a central gelatinous core (Nucleus pulposus, NP) and an external network of collagen fibers (Annulus fibrosus, AF). Unfortunately, IVD can degenerate, causing a debilitating disorder with a considerable socioeconomic impact. Conventional treatments are symptomatic, and new bio-inspired therapies are necessary to overcome IVD degeneration. Recent approaches to engineering a functional IVD model as an alternative to animal experiments are limited and fail to recapitulate the IVD complex organization. Here, we report a three-dimensional (3D) bioprinted IVD model containing IVD cells that can be used in fundamental and translational research and may contribute to the discovery of novel therapeutic strategies.

METHODS: An in-house 3D extrusion bioprinter specially designed for this project was developed. A 3D model was created from a histological section of sheep lumbar IVD. The biomaterial ink (gelatin (7% (w/v)), hyaluronic acid (0.2% (w/v)), alginate (0.6% (w/v))) printability was optimized by varying the printing pressure (0.4-1.1 Bar), flow rate (2-10 mm.s⁻¹) and nozzle type (needle or tip). Then, NP and AF cells were harvested from 4-month-old lambs (N=3), mixed with the biomaterial ink (5*10⁵ cells/mL), and bioprinted. Cell viability (Live/Dead assay), shape (actin immunostaining), and cell distribution (confocal microscopy) were evaluated for up to 21 days of culture.

RESULTS: Using the bioprinter, we successfully set up an analytical model to predict the width of the bioink filament obtained after extrusion as a function of the printing conditions (pressure, flow rate, and nozzle type). This model was used to determine the optimal printing parameters for the IVD construct. The IVD model with fluorescently labeled NP and AF cells was then printed. By confocal microscopy, we observed a homogeneous spatial distribution of the cells, which was maintained for up to 21 days of culture. We also noticed a 25% decrease in the construct height after 21 days of culture, thus modifying the cell distribution, with more cells at the bottom of the construct. Finally, a live/dead assay showed that the cell density was maintained for 21 days, with most cells exhibiting a round shape.

DISCUSSION & CONCLUSIONS: We demonstrated the printability of the biomaterial ink used and the fidelity of the bioprinting process, with the spatial distribution of the cells mimicking an IVD architecture. Future studies will include the deposition of de novo extracellular matrix components and then ex vivo validation of the IVD model.

Keywords: 3D printing and bioprinting, Intervertebral disc / spine and their disorders

PP-075

Building a 3D printed osteocytic network by differentiation of primary human osteoblasts towards construction of a 3D printed in vitro bone model

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INTRODUCTION: Osteocytes are the most abundant bone cell species comprising 90-95 % of all bone cells. Nevertheless, due to their poor availability and their inability to proliferate, human primary osteocytes are rarely applied for in vitro studies, which are in most cases conducted with immortalised rodent osteocyte cell lines. In vitro bone models with close proximity to the clinical situation should optimally comprise human cells. Bioprinting is a versatile tool to generate heterogeneous and functional tissue/organ models with a spatially defined cell distribution. The aim of the study is building a 3D printed osteocyte network by differentiating primary human osteoblasts as a basis for a 3D printed in vitro bone model.

METHODS: Human osteoblasts, isolated from femoral heads as described before (1) were encapsulated into alginate-based inks at different cell densities for 3D bioprinting, here, alginate/methylcellulose/plasma (2), alginate/methylcellulose/egg- white powder and alginate/methylcellulose/gelatin inks were applied. Cell-laden constructs were fabricated by an extrusion-based bioprinter (Bioscaffolder 3.1. Gesim), cross-linked with Ca²⁺ and cultivated for two weeks under low serum conditions. Constructs were analysed by fluorescence microscopy after LIVE/DEAD staining as well as staining of cytoskeleton/nuclei and immunostaining for dentin matrix protein 1 (DMP1).

RESULTS: Osteoblasts showed high viability over two weeks in all examined inks. The cells were able to differentiate into osteocytes as confirmed by dendritic morphology as well as positive immunostaining for DMP1. The cells also secreted osteocalcin, as detected by ELISA.

DISCUSSION & CONCLUSIONS: Bioprinting requires very high cell numbers. Therefore, the application of primary human osteocytes, directly isolated from bone, is not feasible. However, the transition of osteoblasts to terminally differentiated osteocytes was successfully performed with support of the inks, which could provide a suitable ECM-mimicking microenvironment for the formation of dendrites. The generated 3D constructs with osteocyte network offer the opportunity to establish a biomimetic 3D printed in vitro bone model. Future studies will involve bioprinting of spatially separated osteoblasts and osteoclasts, along with terminally differentiated osteocytes in single constructs, using multi-channel and core-shell printing techniques.

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Keywords: Bone and bone disorders (osteoporosis etc), Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-076

Continuous 3D printing of Gelatin/Oxidized Alginate scaffolds with gradient porosity for bone tissue regeneration applications

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INTRODUCTION: The transition between cortical and cancellous bone layers is characterized by gradient porosity and pore sizes [1]. Investigation of extracellular matrix formation on the transition border between the layers is of high interest for bone tissue engineering [2]. Here, we describe the method of fabricating gradient-porosity Gelatin/Oxidized Alginate cryogel scaffolds using continuous extrusion 3-D printing for in-vitro and in-vivo applications in bone tissue regeneration.

METHODS: Three biopolymer inks, varying in gelatin and oxidized alginate concentrations, were printed sequentially in a contact mode, layer-by-layer, in a rectangular spiral (1 cm on each side), followed by crosslinking with a 0.1% glutaraldehyde solution and freeze-drying. Printing accuracy was evaluated for each ink. Scaffold morphology was visualized with a scanning electron microscope. Mechanical properties, swelling capacity, and degradation rate were investigated. The scaffolds were seeded with rat-derived mesenchymal stem cells and incubated in osteogenic cell media for four weeks. The cells' viability and migration were observed under a confocal microscope, and differentiation into osteoblasts was assessed by defining the amount of alkaline phosphatase (ALP) and dimethyl phthalate (DMP). The tissue formation was verified by histological analysis with H&E, alizarin red, and Masson's trichrome.

RESULTS: The inner gradient structure starts with the layer of small diameter pores of 20-50 μm , ascends to 100-150 μm , and ends with the macroporous layer of pores $> 200 \mu\text{m}$. The layers have a graded porosity of 11 to 90%. The scaffold showed high elasticity and an optimal degradation rate. The cells' attachment and viability were above 80% and 87%, respectively. The osteoblasts grew better at the bottom layer, where the highest deposition of calcium and collagen was found. ALP and DMP activity slightly fluctuated but overall remained constant throughout the incubation period.

DISCUSSION & CONCLUSIONS: It was shown that the porous gradient structure supports the attachment of cells to the walls of small pores, osteogenic differentiation in medium-sized pores, and mineralization and collagen production in the macroporous layer. The method is advantageous because of its simplicity and ability to precisely control the pores' size and gradient thickness.

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Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)

PP-077

3D-Bioprinted GelMA Scaffold with ASCs and HUVECs for Engineering Vascularized Adipose Tissue

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INTRODUCTION: The goal of tissue engineering is to reconstruct parts of injured tissues and to solve the organ donation shortage. However, a major limiting factor in the field has been the difficulty of distributing oxygen and nutrients inside three-dimensional (3D) tissue constructs, which severely restricts the size of the tissue area that can be engineered. Vascularization inside tissue engineering scaffolds could be a solution.

METHODS: In this study, we compared the effect of three different blood vessel culturing systems using the 3D-bioprinted gelatin-methacrylate (GelMA) hydrogel construct as our scaffold. We examined the viable blood vasculature created by human adipose tissue-derived stem/stromal cells (ASCs), human umbilical vein endothelial cells (HUVECs), and a co-culture of ASCs and HUVECs.

RESULTS: In vitro results show that after a 21-day culture the number of live blood vessel cells in the co-culture of the ASCs and HUVECs is the highest among the three approaches tested. Additionally, tubular structure is the most abundant in the co-culture approach. In vivo results show that blood vessels are present in mice after an implant of a hydrogel containing the HUVECs as well as the co-culture of the ASCs and HUVECs. However, blood vessel density is, again, highest in the co-culture method.

DISCUSSION & CONCLUSIONS: These results indicate that the 3D-bioprinted GelMA hydrogel co-culturing system could be a promising biomaterial for large tissue engineering applications.

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Keywords: Biofabrication, Biomaterials

PP-078

Gellan gum fluid gels as a suspension media for 3D bioprinting

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INTRODUCTION: Recently, self-healing viscoplastic fluids [1], which we refer to as suspension media, have been employed in combination with extrusion 3D printers. These suspension media have garnered interest as they provide the required structural support to constructs printed from hydrogels which are soft and high in water-content. In our work, we develop a structured fluid gel, comprised of polysaccharide particles and investigate the influence of such gels on the 3D bioprinting process. Specifically, we compare printing in our developed fluid gels to conventional layer-by-layer 3D printing in air, in order to gain a greater understanding of how the microarchitecture of our fluid gels can be used to enact control over the 3D printed construct shape.

METHODS: Polysaccharide fluid gels were developed using gellan gum, a clinically approved biomaterial. By applying a flow field to solutions containing very low concentrations of this polymer (0.1 and 0.5 % w/v) as they passed through their gelation temperature (~ 42 oC), gel particles in a continuous fluid matrix were obtained. Shear amplitude sweeps and alternating shear stress tests were conducted on the fluid gels using a Kinexus rheometer. Extrusion-based bioprinting (filament and droplet printing) in air and in the fluid gels was performed using a Cellink BioX printer equipped with pneumatic and electromagnetic droplet (EMD) printheads.

RESULTS: Brightfield microscopy of gellan gum fluid gels indicated that the particles were heterogeneous in size and structure, with the majority of particles exhibiting hairy protrusions extending from the particle body. Amplitude sweeps indicated that these gels had exceptionally low yield stress values, in the range of approximately 0.5-5 Pa, while the self-healing time of these gels was found to be around 3 s regardless of gel thermal history. Printed filaments were found to have a convoluted shape when printed in the fluid gels compared to air, while printed droplets were found to have a higher surface roughness, and cylindrical shape in the fluid gels.

DISCUSSION & CONCLUSIONS: Our results indicated that gellan gum fluid gels could be developed to have viscoplastic and self-healing behavior providing structural support to 3D printed hydrogels. These fluid gels exhibited good thermal stability at physiological temperatures due to the melting temperature of the gellan particles being higher than 37 oC. A reduction in surface tension when printing in fluid gels compared to printing in air was found to significantly affect both the shape and surface roughness of printed structures.

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Keywords: 3D printing and bioprinting, Biomaterials

PP-079

Functional outcomes of bio 3D nerve conduit transplantation for peripheral nerve injury

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INTRODUCTION: World's first clinical study to transplant the bio 3D nerve conduit for peripheral nerve injury in November 2020. The outcomes to evaluate the efficacy of the bio 3D conduit transplantation was required for clinical trial. The purpose of this study is to extract outcomes of functional analysis that can evaluate the effectiveness of bio 3D conduit transplantation.

METHODS: The clinical study included nerve injuries distal to the wrist joint. Therefore, we selected sensory and motor nerve evaluation methods for the evaluation of sensory and motor nerves distal to the wrist joint.

RESULTS: For the sensory nerve evaluation, the Semmes-Weinstein monofilament test and static two-point discrimination test were selected. The motor nerves were evaluated by manual muscle strength testing and the Perfect O test for the median nerve, and the ulnar nerve was evaluated by the presence or absence of the Frohman's sign. The patients were also interviewed to determine whether or not they had returned to work.

DISCUSSION & CONCLUSIONS: Clinical scores and diagnostic imaging are often used to determine the efficacy of transplantation of regenerative medicine products. Although these methods are effective, it is necessary to conduct functional evaluation in order to increase patient treatment satisfaction after transplantation. Outcomes based on the functional evaluation methods extracted in this study are useful as a method for evaluating the efficacy of transplanting regenerative medical products.

ACKNOWLEDGEMENTS: We acknowledge the staffs of Institute for Advancement of Clinical Translational Science and Department of Rehabilitation in Kyoto University Hospital to make the protocol of clinical trial.

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Keywords: 3D printing and bioprinting, Translation and commercialisation (inc. clinical trials and regulatory approval)

PP-080

Manuka Honey-enriched photocurable gellan gum for biofabrication of articular cartilage constructs

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INTRODUCTION: Bioprinting is radically changing regenerative medicine, allowing the manufacture of tissue-engineered constructs with appropriate control over spatial variations and capability of precise deposition of biomaterials, cells and biological molecules. One of the main challenges is to identify bioinks able to fulfill the requirements for reproducible additive manufacturing and the biological requirements for the type of tissue required. Hydrogel-based bioinks are the main formulations exploited for articular cartilage (AC) modelling, due to their tissue similarity in terms of morphological and mechanical properties(1). Herein, we propose a photocurable gellan gum methacrylated (GGMA) based bioink suitable for extrusion-based bioprinting to generate bioprinted AC tissues constructs. We exploited a nature-inspired strategy, adding Manuka Honey (MH) to the GGMA composition (GGMA-MH)(2).

METHODS: H-NMR, XPS and FTIR were performed to assess the methacrylation efficiency. Bioinks formulations composed of GGMA or GGMA-MH and mesenchymal stem cells differentiated in chondrocytes (Y201-Cs) were extruded to obtain a grid-shaped construct with Roket INVIVO 4D2 bioprinter. SEM analysis was performed to assess the porosity and to evaluate cells morphology and organisation. Water uptake and mechanical properties were assessed. Cells viability was monitored straight after the printing process, and over 7 days of culture via Live/Dead, Immunostaining and ATP production. Gene expression analysis (ACAN, COL2A1 and SOX9), mucopolysaccharides quantification and histological analyses (H&E, Picrosirius Red, Alcian Blue) were performed to evaluate the chondrogenic potential of the bioinks formulation.

RESULTS: GGMA methacrylation efficiency was assessed with H-NMR, XPS and FTIR. GGMA/Y201-C and GGMA-MH/Y201-C bioinks printability were suitable. The chemical crosslinking occurred via UV irradiation, while the physical crosslinking occurred with DMEM/F12 onto the printing bed. Both compositions showed porous interconnected structure, with pore size in the range 50-250 µm, optimal for nutrition supply and diffusion, together with an high hydrophilicity (by water uptake analysis), and suitable mechanical properties for AC regeneration. MH incorporation was beneficial for cells printability, viability and chondrogenic potential, as demonstrated by cell viability assays, as well as for cells chondrogenic potential, by histology, gene expression analysis and mucopolysaccharides quantification.

DISCUSSION & CONCLUSIONS: Y201-C loaded GGMA and GGMA-MH represent potential bioink formulations for AC-like construct. The bioprinting process, with the proper choice of parameters, enabled us to print with spatial and microscale resolution in a rapid and continuous manner, without affecting cell viability. MH led to the obtainment of an antimicrobial environment, more viscous formulation, and an optimal cells distribution pattern, inducing the Y201-C to exhibit maximum

biosynthetic ability.

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Keywords: 3D printing and bioprinting, Cartilage / joint and arthritic conditions

PP-081

3D bioprinted neural construct for the *in vitro* modeling of spinal cord

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INTRODUCTION: Spinal cord injury is a debilitating neurological condition that leads to permanent neural damage with consequent impairment of sensory and locomotor functions. Unfortunately, effective treatments are not yet available for a successful bridging of the injured segment and complete recovery of nervous functionality¹. Indeed, most of the experimental drugs are proven to be unsatisfactory during clinical trials, partially due to the weakness of actual preclinical models in replicating the pathophysiology of the central nervous system (CNS)². In this context, 3D bioprinting technology has gained increasing attention in neural research, as it allows the creation of complex engineered structures which mimic the neural microenvironment. This work aims to develop a three-dimensional neural construct for the *in vitro* modeling of the spinal cord. Specifically, the optimization of biomimetic photo-crosslinkable hydrogels has been reported, intended for the bioprinting of the most represented cell phenotypes found in the spinal cord and the establishment of a multicellular 3D culture system.

METHODS: Gelatin Methacryloyl and Thiolated Hyaluronic Acid were selected as the main constituents of the hydrogels because of their high flexibility and suitability for the printing process. Different concentrations of the two polymers were investigated to obtain a fast gelation rate and improve hydrogel extrudability in cell-friendly conditions. Hydrogel characterization was conducted in terms of rheological properties and printability. In addition, preliminary *in vitro* studies were performed by encapsulating a motor neuron cell line (NSC-34) and a neural stem cell line (NE-4C) into specific hydrogel compositions. Lastly, the cell-loaded hydrogels were printed, and the obtained constructs were cultivated for up to seven days.

RESULTS: Rheological studies showed that the formulated hydrogels display shear-thinning properties and the presence of a rapid sol-gel transition after visible light exposure. According to morphological analysis, the printed constructs exhibited regular and well-defined geometry and macroporosity, thus confirming hydrogel extrudability and shape fidelity. Moreover, *in vitro* assays demonstrated bioink cytocompatibility and revealed that the printing process does not affect cell viability and behavior.

DISCUSSION & CONCLUSIONS: These results suggest that the optimized bioinks may be potential candidates for the bioprinting of a neural-like construct with high-resolution and good structural stability. Future investigations will be focused on the inclusion of topographical and biochemical cues to directly induce the differentiation of encapsulated cells into CNS cell subtypes.

ACKNOWLEDGEMENTS: This project has received funding from Fondazione CRT (grant RF 2020.1801).

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Keywords: Biofabrication, Nervous system (brain-central-peripheral / disorders)

PP-082

3D bioprinted constructs comprising gellan gum/PVA bioinks enriched with nanohydroxyapatite for bone tissue regeneration

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INTRODUCTION: Bioprinting is one of the most versatile 3D printing techniques in the field of tissue engineering, combining cells and biomaterials into constructs with desired geometry. This in turn leads to an increasing demand for the development of new combinations of biomaterials and biological constituents in order to produce biomimicking devices, optimal for tissue regeneration [1]. In this work, we prepared two different compositions of bioinks comprising gellan gum (GG) and polyvinyl alcohol (PVA) [2] with nanohydroxyapatite (HAP) [3], to assess their mechanical properties, and their osteogenic potential when mixed with MC3T3-E1 pre-osteoblasts.

METHODS: Four different bioinks were prepared by varying ratios of GG and PVA, with and without the presence of a standard concentration of HAP, by mixing with MC3T3-E1 pre-osteoblasts cell suspensions and crosslinked with 1% w/v CaCl₂ solution. The biological evaluation of the bioinks includes staining with Live/Dead, DAPI/actin, collagen and alizarin red. Moreover, the bioinks have been mechanically characterized by examining their biodegradation rate and their rheological properties.

RESULTS: At day 7, all bioinks had evident extracellular matrix formation, with the HAP containing bioinks depicting greater levels. Regarding the biodegradation rate, the GG-2 bioink display values between 14% and 28%, the GG-2.8 7% and 23%, the HAP/GG-2 9% and 17% and the HAP/GG-2.8 5% and 16% on days 7 and 21, respectively. The rheological analysis showcased that the presence of HAP only slightly affected the examined quantities. Specifically, the yield points ranged between 11 and 28 kPa for the GG-2 and HAP/GG-2.8 respectively, with the dynamic frequency sweep analysis validating the G' and G'' values. Finally, all bioinks retained a recovery rate of at least 89% of their viscosity levels after the application of a 200% strain.

DISCUSSION & CONCLUSIONS: Novel bioinks containing gellan gum, PVA, and hydroxyapatite were constructed to assess their biomechanical traits. The 3D bioprinted constructs were mixed with pre-osteoblasts and were evaluated for bone tissue growth. Their rheological properties support their excellent bioprintability efficiency, while their biocompatible attributes promote bone regeneration.

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Keywords: 3D printing and bioprinting, Biofabrication

PP-083

Micro-IAIver: Artificial intelligence-powered drug screening platform for 3D-bioprinted human liver microspheres

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INTRODUCTION: non-alcoholic fatty liver (NAFL) is characterized by > 5% steatotic hepatocytes. Its more aggressive form is known as non-alcoholic steatohepatitis (NASH). This latter form is characterized by extended liver inflammation and often accompanied by fibrosis which may progress to cirrhosis and hepatocellular carcinoma (HCC). There are no drugs for NAFL/NASH treatment because of the high failure rate of the clinical trial phases. The standard pipeline for identifying drug candidates lacks accuracy and reproducibility, especially in the preclinical phase. Many in vitro models have been developed to recapitulate the in vitro pathophysiological conditions of NAFL/NASH. However, these models are difficult (i) to replicate, (ii) to scale to an industrial setting, and (iii) to identify a clear outcome. Here, we use UniINK, a patented 3D printer ink employed for cell encapsulation. We used this technology to recreate a mini-liver using hepatocytes, hepatic stellate cells, and Kupffer cells in a high-throughput manner, minimizing the operator's input. Upon fat treatment, UniINK presents the main signatures of NAFL/NASH in a time-dependent manner, including fibrosis accumulation. These features are identified, analyzed, and interpreted by a machine learning approach to calculate the therapeutic index of drug candidates.

METHODS: UniINK is collagen-based ink further crosslinked by tannic acid. HepaRG (human hepatocytes), LX-2 (human stellate cells), and THP-1 (human monocytes) are encapsulated in spheroids using a 3D bioprinter and challenged with a mixture of oleic and palmitic acids. At the end of the experiment, the spheroids are passed through a flow-based tomographic microscope and interrogated one-by-one for features of hepatocyte death and HSC, THP-1 activation.

RESULTS: We developed a robust 3D bioprinting method that fabricates 48k micro-livers/hour with an average cell density of 3M cells/spheroid at €0.80/spheroids. The spheroids are incubated for up to one month, showing no necrotic area and high viability for untreated cells, whereas fat and fibrosis accumulation and cell death in fat-treated conditions. The features are classified using K nearest neighbour (k-NN), support vector machine (SVM), and decision tree to build and implement the dataset. Deep learning networks, such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs), are employed to automatically extract features, group the data, and recognize patterns to make decisions based on the beneficial effects of drug treatment.

DISCUSSION & CONCLUSIONS: micro-IAI-ver allows: 1) to reduce the variability associated with the operator; 2) to be scalable at the industry level; 3) to spot early signs of fibrosis accumulation.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: 3D printing and bioprinting, Disease models

PP-084

Parameters Optimization of a Collagen-based Scaffold Loaded with Nanocarriers For Growth Factor Controlled Delivery and Bioengineered with Human Stem Cells

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INTRODUCTION: In the last decade, despite the potential of 3D bioprinting technology in manufacturing hydrogel-based scaffolds had been widely explored, the next big challenges are the development of advanced nanocarriers able to be directly incorporated into bio-inks for promoting more accurate drug delivery (1,2,3,4,5). Nowadays, current technology enables us to finely tune scaffold shape and porosity thanks to bioprinters, choose the most suitable hydrogel for the specific application, incorporate nanocarriers in the master mix, and seed patients' staminal cells (6). Some problems arise in this pipeline: how many particles need to incorporate in the gel, which cell density is better to use, and how dense the hydrogel should be. In addition to that, using cells directly from patients could lead to more precise information and lastly, to personalized therapy (7).

METHODS: Firstly, different concentrations (ranging from 3 to 10 mg/mL) of Methacrylated Collagen (ColMA) were investigated to find the best printability; then, various amounts (from 10 up to 150 µg/mL) of rhodamine-loaded PLGA nanocarriers were integrated to better understand how they interfere with the printing process or cross-linking. After that, Tendon Progenitor Stem Cells (TPSC) were incorporated into the master mix (containing ColMA and PLGA-nanoparticles), to study and optimize cells vitality and printability. Finally, PLGA-Transforming growth factor-β1 (TGF-β1)-loaded nanoparticles were added to ColMA, and TPSC, bioprinted, and cultured for up to 14 days to study the differentiation pattern of TPSC into mature tendon cells.

RESULTS: ColMA at 6 mg/mL resulted in the best concentration able to mimic ExtraCellular Matrix (ECM) and even the highest concentration of PLGA-rhodamine-loaded nanoparticles tested did not obstruct the printing process. When cells were incorporated in the master mix, Live/Dead assay showed that the optimal cell density was about 1 million/mL. After 14 days of incubation of the scaffold made with ColMA, PLGA-TGF-β1-loaded nanoparticles, and TPSC, Immunostaining, Microtome procedure, and PCR displayed that TPSC differentiated toward tendon cells and ColMA provided a 3D environment suitable for this kind of application.

DISCUSSION & CONCLUSIONS: The presented study highlighted the capability of ColMA to reproduce an ECM-like environment suitable for TPSC seeding; it also determined the better concentration for the ColMA-scaffold, the ideal concentration of PLGA-nanoparticles to be printed with cells, the optimal ratio to effectively deliver the drugs inside and, ultimately, that this is a valid and complex enough support to be a test-bench for personalized therapy.

ACKNOWLEDGEMENTS:

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Keywords: 3D printing and bioprinting, Personalised medicine

PP-085

Development of a Polyplexes-based miRNA Delivery System in a 3D-Bioplotting Osteoarthritis Cellular Model

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INTRODUCTION: To date, there are no satisfactory therapies for Osteoarthritis (OA), but only palliative methods to reduce chronic pain: therefore, more understanding and knowledge of OA mechanisms and related pathways are needed [1]. This work aimed to investigate the role of micro-RNAs(miRNAs)-loaded polyplexes within a bioprinted cell-laden Gellan Gum Methacrylate (GGMA) in-vitro model [2], simulating OA disease conditions. miRNAs were the molecule of choice due to their established role in regulating gene expression by inhibiting mRNA translation and/or facilitating mRNA degradation [3]. Branched polyethyleneimine-graft-poly(ethylene glycol) (PEI-g-PEG), was the polymer used to encapsulate miRNA thanks to its positive superficial charge, high transfection efficiency, excellent encapsulation capability of oligonucleotides, low cost of production and convenient storage solutions [4].

METHODS: PEI-g-PEG-polyplexes loaded with miRNA-140-5p were manufactured and characterized for their size and ζ -potential using Dynamic Light Scattering (DLS). In this first phase, the N:P ratio and the concentration of PEI in relation to PEG were the main focus. Then, their cytocompatibility was tested by using immortalized Y201 stromal Mesenchymal Stem Cells (MSCs). Lastly, 3D GGMA hydrogels were bioprinted, encapsulated with cells, and polyplexes, and cultured for up to 14 days. To simulate OA, cells were nurtured with a cocktail of cytokines. Healthy condition was used as control.

RESULTS: DLS showed that the size of the polyplexes ranged from ~90 nm to ~1000 nm and ζ -potential ranged from ~-1 mV to ~45 mV. Of the two concentrations of PEI-g-PEG studied (0.1% and 0.9% w/v), the lowest along with an N:P ratio of 7, proved to be non-cytotoxic and was able to deliver miRNAs inside cells (as shown by Live/Dead and immunostaining results). Results coming from Hematoxylin and Eosin (H&E), and Alcian Blue stainings confirmed that the pathological media successfully simulated OA conditions. In addition to that, they also showed that polyplexes stimulated the production of ExtraCellular Matrix (ECM), and glycosaminoglycans.

DISCUSSION & CONCLUSIONS: Overall, this model well reproduced an in-vitro 3D model of OA conditions as shown by the staining used; PEI-g-PEG is believed to be a cheaper and more reliable alternative to the current gold-standard (chitosan-based nanoparticles), and the promising loading capabilities and easy-to-use protocols are the main advantages. Polyplexes made with PEI-g-PEG also proved to be easily incorporated in GGMA and did not interfere with the bioplotting process. Nevertheless, the exact threshold above which it becomes cytotoxic is yet to be fully assessed.

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Keywords: Gene therapy, Cartilage / joint and arthritic conditions

PP-086

High-fidelity 3D bioprinting of mechanically stable cell-laden scaffolds for bone tissue engineering

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INTRODUCTION: Polymeric scaffolds can be used to mimic essential extracellular matrix (ECM) complexity and cellular processes of the bone tissue and create three-dimensional (3D) in-vitro models to study bone osteogenesis and pathology. Commonly, collagen organization and characterization are overlooked during the development of bone grafts or bone tissues, mainly focusing on the ability of cells to mineralize the scaffold. 3D printing (3DP) is used to create a polymeric scaffold by including primary human cells directly in the bioink. This approach allows accurate deposition of biomaterials in predesigned architectures granting better control and guidance of the substrate and enabling the formation of a fibrous bone network. Here, we describe 3DP of stable hydrogel-based cell-laden scaffolds with dynamically changing architecture for creating 3D engineered bone tissues.

METHODS: Human mesenchymal stem cells (hMSCs) were mixed with the bioinks containing various Alginate (Alg), Gelatin (Gel), and methacrylated Gel (GelMA) concentrations. During 3DP (Cellink BIOx), a lattice-rod model was extruded at the pressure of 30kPa, and the system temperature was kept between 10-15 °C. The scaffolds were photo-crosslinked with UV and 0,25M CaCl₂ solution. An osteogenic culture medium was used to promote hMSCs differentiation into osteoblasts, and the resulting scaffolds were characterized temporally for up to 6 weeks. The rheological properties and printability of the bioinks were optimized to provide high print fidelity. Mechanical properties and morphology of the printed scaffolds in culture were systematically investigated. Qualitative and quantitative characterization of formed tissue was studied using histology (H&E, Sirius Red, Alizarin Red) tests.

RESULTS: Rheology experiments revealed that Gel assisted in the modulation of the bioink viscosity and, enabling the bioprinting of a high-fidelity stable scaffold with printing accuracy >90%. Formulations with GelMA content of less than 5 (w/v %) lost about 70% of their original after several days. Samples with 5 (w/v %) of GelMA demonstrated clear dendritic formation and increased ECM deposition and mineralization of the scaffold after 6 weeks in culture. Collagen staining of these scaffolds demonstrated the tendency of uniform organization and alignment of collagen fibers mostly along the printed rods.

DISCUSSION & CONCLUSIONS: In summary, these data demonstrate that GelMA concentration of 5% was suitable for bioprinting and creating stable constructs for up to 6 weeks. The scaffold's morphological and histological analysis verified the presence of a collagen matrix that aided in the establishment of viable 3D in vitro bone tissue in the printed scaffold.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-087

Extrusion-based 3D bioprinting from bench to bedside applications in bone tissue engineering using platelet rich plasma and scaffolding materials

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INTRODUCTION: Extrusion-based 3D bioprinting is a promising technique to create patients' specific implants for bone reconstruction, however, the current bone tissue engineering scaffolds produced by this method lack osteoinductivity which is crucial for bone reconstruction.

METHODS: In this study, we utilized Platelet Rich Plasma (PRP) as bioactive bone-inducing content in our proprietary bioink for bone formation. In addition, polycaprolactone and tricalcium phosphate were used as the scaffold material to provide mechanical support. The structure of patients' specific implants was created based on their CT images, engineers' designs, and the surgeon's input in performing surgeries. After the concurrence from the engineering and clinical team, patients' specific implants were printed in a GMP lab with an ISO 5 environment.

RESULTS: The printed constructs showed a very low level of endotoxin with zero occurrences of contamination. Three cases of clinical trials were conducted using these patients' specific implants. Results showed excellent osteointegration and osteoinduction between implants and host tissues.

DISCUSSION & CONCLUSIONS: All patients could release from the hospital after one week of observation in the hospital without any complications. This bench to bedside application of 3D bioprinting and regenerative medicine is promising and should be widely promoted in more hospitals.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: 3D printing and bioprinting, Advanced therapy medicinal products

PP-088

Microfluidic-based 3D bioprinting for fabrication of helical fibres

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INTRODUCTION: 3D extrusion-based bioprinting has emerged as a promising technology for development of advanced and viable organs and tissues and functional 3D in vitro disease models [1,2]. However, these developments have failed to recapitulate the heterogeneity and complexity (e.g. helical structures) of organs and tissues. Recent studies [1,2] have demonstrated the potential use of microfluidic-based 3D printing by allowing more control and precision over the organisation of biomolecules, cells and material thus, enabling fabrication of complex 3D constructs. Herein, we developed an agile manufacturing pipeline based on COntinuously Varied Extrusion (CONVEX) [3] design approach in extrusion-based printing to develop integrated 3D-printed microfluidic chip nozzles, based on microfluidic mixers and hydrodynamic flow focusing components with the potential to fabricate complex helical fibres.

METHODS: FullControl GCode designer software [4] was used to extrude a single layer of acrylonitrile butadiene styrene (ABS) with zigzag passive mixer component using an Ender 3 3D printer. The ABS channels were cast into polydimethylsiloxane (PDMS) before flushing with acetone. To achieve co-axial hydrodynamic flow focusing of calcium chloride-Pluronic solution by 2wt% sodium alginate solutions, the calcium chloride channel had a smaller diameter than sodium alginate channels.

RESULTS: Complex passive mixer region zigzag region has enabled rapid on-fly mixing of two fluids, while achieving flow-focusing capabilities through cross junction design, resulting in formation of helical fibres. 3D-Helical fibres are among the most interesting and innovative structures in nature, representing an emerging group of materials with distinct unique spiral geometry and multiple excellent functionalities. However, their fabrication at micro-scale level remains a challenge. This work presents an innovative and highly adoptable 3D-printed fluidic chip system, with the functions of consecutive spiralling for scalable generation of helical fibres. The generation of helical structures can be precisely optimised by varying the flow rates; therefore the length, diameter and pitch of the helical structures are highly controllable.

DISCUSSION & CONCLUSIONS: An agile manufacturing pipeline has been developed to fabricate novel microfluidic chip nozzles suitable for 3D bioprinting of complex tissues and organs. Microfluidic chip nozzles allowed dynamic and precise control of the diameter of core/shell hydrogels. Future work will integrate the microfluidic chip nozzle on a 3D printer to fabricate complex 3D structures containing cells.

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Keywords: 3D printing and bioprinting, Additive manufacturing

PP-089

Tunicate nanocellulose hydrogels for tissue bioengineering: cytotoxicity, inflammation and in vivo biocompatibility assessments.

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INTRODUCTION: We assessed the cytotoxicity, inflammatory response and in vivo compatibility of tunable, hydrogels based on cellulose nanofibrils (TUNICELL) isolated from tunicates grown in Norwegian fjords. TUNICELL, processed in a GMP compliant cleanroom, has high purity (99%), crystallinity (89%), is sterilized, and has endotoxin levels below 0.5 EU/ml, meeting medical requirements.

METHODS: For cytotoxicity and inflammation experiments, enzymatically prepared (ETC), TEMPO-oxidized (TTC) and carboxymethylated (CTC) versions of TUNICELL were used, with samples prepared for both direct and extract tests according to ISO10993-12. Samples were assayed using mammalian HepG2 and THP-1 cell lines. In vivo biocompatibility assessments were run using ETC discs over a 90-day period in immunocompetent Wistar rats with surgical ePTE surgical meshes as controls.

RESULTS: All TUNICELL samples tested exhibited cell viabilities >70% in both cell lines as assayed by WST-1 reduction and cell death <30% as assayed by presence of released LDH in the culture medium. THP-1 cells always exhibited less than 10 pg/ml of pro-inflammatory cytokine TNF α secretion when exposed to the TUNICELL test samples. All Wistar rats thrived throughout the study period, and upon explantation, the ETC discs were easily retrieved, cohesive, and macroscopically well integrated in the surrounding tissues with no macroscopic signs of inflammation or other adverse reactions. ETC implants generally displayed abundant neovascularization, infiltrated connective tissue, and a higher grade of biointegration as compared with the control ePTE group.

DISCUSSION & CONCLUSIONS: In vitro cell culture assays showed that all 3 TUNICELL hydrogel versions were non-cytotoxic according to ISO-10993-5 criteria and were not pro-inflammatory according to ISO-10993-20. In Wistar rats, semi-quantitative ISO10993-6 assessments at both 30 and 90 days resulted in a score of non-irritant as compared to ePTE controls. Results from these studies show that from a biological and structural perspective, TUNICELL hydrogels are well suited to regenerative tissue engineering applications. The penetrating neovascularization evident in in vivo assessments of the TUNICELL constructs is very interesting and will potentially bring tissue engineering a step closer to clinical translation.

Keywords: Polymers - natural / synthetic / responsive, Biomaterials

PP-090

Bioprinted 3D embryo implantation model

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INTRODUCTION: Despite the immense progress in assisted reproductive technologies, success rates stand at approximately 30% per embryo transfer. There is a great need for a proper model that will simulate implantation and allow for the examination of treatment options (1). We aim to develop a 3D bioprinted cell culture system consisting of both epithelial and stromal endometrial cells cultured under a specific hormone regime. Using a spheroid as an embryo surrogate, we strive to examine implantation under different manipulations to improve implantation success rates.

METHODS: RL95-2 was used as epithelium, THESCs as stroma, and JAR spheroids as embryo surrogates.

Epithelial cells or stromal were encapsulated separately in alginate and bio-printed under specific and optimized conditions. Cell viability was confirmed by Presto Blue assay. Bilayer constructs consisting of a bottom-stromal and top-epithelial layer were printed. Bio-printed constructs were treated with a sequential hormonal treatment: 10-8 M estrogen for 2 days followed by 10-8 M estrogen + 10-7 M progesterone for 3 days. Controls were treated with the solvent ethanol. Cells were examined for protein expression by Western blot (WB). Membrane-stained Jar spheroids were placed upon the stained bilayer constructs and incubated for 48h to allow invasion. Hydrogels were fixed, stained with Hoechst nuclear staining, and imaged using confocal imaging.

RESULTS: RL95-2 and THESCs constructs showed over 80% viability for over a week of culture. Live cell imaging of the bilayer hydrogel constructs showed separate cell layers.

WB analysis of E-Cadherin indicated that E-cadherin expression is significantly higher in the estrogen-treated constructs compared to controls, at day 2. ER α expression levels were shown to be lower in the sequential hormonal-treated constructs compared to controls, at day 5.

Imaging of the Jar invasion test showed Jar cells embedded within the epithelial upper layer, and also present in the stromal lower layer of the printed bi-layer hydrogel construct.

DISCUSSION & CONCLUSIONS: We successfully designed bilayer bioprinted hydrogels simulating the inner stroma and outer epithelial lining. The hydrogels demonstrated hormone responsiveness, consistent with the known effects of estrogen and progesterone on E-cadherin and ER α expressions, respectively. Jar spheroids successfully infiltrated through the epithelial layer to the stromal layer in the printed constructs, demonstrating embryo implantation.

ACKNOWLEDGEMENTS: Azrieli College of Engineering, Jerusalem (TTR).

REFERENCES: (1) Stern-Tal D et al; Achache H, Jacobs Catane L, Reich R, Tavor Re'em T. JBE. 2020 Jun 30;14(1):18.

Keywords: In vitro microenvironments, 3D printing and bioprinting

PP-091

Improved alginate bio ink by recombinant spider-silk inspired-biopolymer

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INTRODUCTION: Alginate, a natural linear polysaccharide polymer, extracted from brown seaweed, is extensively applied due to its biocompatibility, all-aqueous ease of handling, and relatively low costs. Alginate easily forms a hydrogel when crosslinked with a bivalent ion, such as calcium. However, Alginate hydrogel holds low mechanical properties and is cell-inert, having no cell-matrix interactions. To overcome these drawbacks and to improve alginate as a bioink for bioprinting, we produced a new alginate matrix combined with spider silk, one of the most resilient, elastic, strong materials known to men, and known to enable cell adherence. The recombinant spider silk biopolymer used in our study (SVX) (1), is synthetically produced, has a sponge-like structure, and is known to be biocompatible and non-immunogenic.

METHODS: The viscosity of the alginate with added amounts of SVX was measured by viscometer. 3D a-cellular and L929 cellular hydrogel models were prepared, and tension/ compression tests were performed by a tensiometer, and stress-strain curves were prepared. Bioprinting was performed by a 3D Allevi2 bioprinter. The spreading ratio was calculated as the width of the printed filament/ the internal needle diameter. Cell viability was measured using a Cell Titer-Glo assay (Promega). Bioprinted cell hydrogels were visualized by light microscope, and stained constructs with Calcein or with Thioflavin S were imaged by a confocal microscope.

RESULTS: Combining synthetic spider-silk, with alginate resulted in higher viscosity, and cell-seeded hydrogels with higher resilience to compression, compared to alginate. SVX-enriched alginate demonstrated a higher degree of printability, in terms of lower spreading ratio at lower pressures, favorable for cell printing, and significantly higher uniformity in spreading ratio at a wide range of setup conditions. Bioprinting of cells within the SVX-enriched alginate bioink resulted in a more homogenous culture with prolonged and higher cell viability, compared to larger, more condensed spheroids with lower cell viability in the alginate bioprinted constructs.

DISCUSSION & CONCLUSIONS: Alginate enrichment with synthetic spider-silk resulted in improved mechanical properties compared to alginate and a higher degree of printability. The more homogenous culture with prolonged and higher cell viability in SVX-enriched alginate bioprinted constructs may be explained by the higher construct stability, but also due to the spider-silk mediated cell adherence.

ACKNOWLEDGEMENTS: Azrieli College of Engineering, Jerusalem (TTR).

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Keywords: 3D printing and bioprinting, Biologics and growth factors

PP-092

Atmospheric pressure plasma-assisted electrospinning of bead-free honeycomb-patterned nanofibers for the ultimate generation of microvascular networks

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INTRODUCTION: Despite all advances in tissue engineering (TE), a fundamental challenge facing biofabricated scaffolds is their poor in vivo vascularization that is essential for cell survival. Therefore, it is vital to design scaffolds possessing properties enabling the formation of vascular networks and exhibiting a high affinity towards blood-vessel forming cells. The mimicry of the extracellular matrix via electrospun nanofibrous scaffolds has shown a superiority over other structures in terms of cell adhesion and other performances. However, the small inter-fiber spaces of nanofibers reduces their porosity thus hampering the vascularization. Therefore, 3D microporous scaffolds were adopted in blood vessel TE. Particularly, scaffolds with honeycomb pores were revealed to imitate the microvasculature network but were deprived from the superior cell affinity afforded by nanostructures. Interestingly, some researchers introduced the possibility of electrospinning honeycomb-patterned nanofibers by a simple self-assembly of the electrospun products. Nonetheless, the low polymer concentration solutions that are needed to trigger this self-assembly always led to the formation of poor quality beaded nanofibers.

METHODS: To overcome this issue and obtain for the first time bead-free fibrous honeycomb structures, a pre-electrospinning treatment of polycaprolactone (PCL) solutions was performed using non-thermal plasma. To do so, a distinctive atmospheric pressure plasma reactor engendering an argon plasma jet with an afterglow in contact with the solution was used. A parametric study of the electrospinning process was first conducted with different solvents systems (chloroform/dimethylformamide, dichloromethane/dimethylformamide and formic acid/acetic acid), PCL molecular weights and concentrations, applied voltages and tip-to-collector distances. Concurrently, the plasma parameters (gas flow rate, applied voltage and plasma exposure time) were delicately tailored to treat the solutions prior to electrospinning.

RESULTS: Results revealed that the solvent system chloroform/dimethylformamide 4/1, PCL weight of 37000 g/mol, PCL concentration of 17.5% w/v, applied voltage of 22.5 kV and tip-to-collector distance of 15 cm led to an optimal fibers self-assembly into clear honeycomb patterns. Moreover, a pre-plasma treatment of all solutions triggered a significant enhancement in the fibers quality with less to no beads. This is attributed to a plasma-induced degradation of the solvent molecules generating new species such as HNO₃ and HCL which enhanced the solution conductivity and electrospinnability. Particularly, a treatment time of 15 s, argon flow rate of 0.5 slm and applied voltage of 8 kV resulted in a well-balanced morphology combining honeycomb with reduced amount of beads.

DISCUSSION & CONCLUSIONS: Overall, innovative high-quality bead-free honeycomb-patterned nanofibers that can be used for an enhanced vascularization in TE applications were generated.

Keywords: Biomaterials, Vascular systems / vascularisation and heart

PP-093

Developing Hierarchical Biomimetic Scaffolds of the Human Trabecular Meshwork

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INTRODUCTION: Glaucoma is the leading cause of irreversible blindness worldwide and is associated with a rise in intraocular pressure (IOP). The trabecular meshwork (TM) is essential for regulating aqueous humour outflow, which directly impacts IOP. Yet, in glaucoma, a drastic reduction in cellularity is associated with blockages of the porous TM resulting in elevated IOP and leading to irreversible vision loss. Synthetic scaffolds that mimic the TM's fine-scale anatomical 3D hierarchical architecture could be utilised as a novel tissue engineered intervention for TM replacement. Here we compare the architecture of the human TM to our artificial replicates and investigate the ability of such scaffolds to support TM cell viability, morphology and infiltration.

METHODS: Poly(ϵ -caprolactone) (PCL; 12%w/v and 20%w/v) and poly(lactic-co-glycolic acid) (PLGA8515; 8%w/v) were dissolved separately in 1,1,1,3,3,3-hexafluoroisopropanol. Two biomimetic TM's were fabricated by first spinning PLGA8515, followed by spinning PCL 12% directly on top (PLGA:PCL) and in a second, separate scaffold electrospinning PCL 20%, followed by PCL 12% (PCL20:12). Scaffold structure was compared to human TM using X-ray computed tomography (X-CT). An immortalised TM cell line (NTM5) was cultured (60,000 cells/cm²) on both scaffolds for 14-days to monitor cell viability (AlamarBlue Assay), cell attachment and morphology (scanning electron microscopy (SEM)), and infiltration (X-CT).

RESULTS: 3D structural analysis of PLGA:PCL and PCL20:12 using reconstructed X-CT images revealed a decrease in fibre size (PLGA:PCL 2.5 $\pm$ 0.4 μ m to 1.7 $\pm$ 0.5 μ m; PCL20:12 3.1 $\pm$ 0.8 μ m to 1.9 $\pm$ 0.4 μ m) and pore size (PLGA:PCL 6.6 $\pm$ 3.1 μ m to 4.2 $\pm$ 2.3 μ m; PCL 20:12 7.3 $\pm$ 2.4 μ m to 5.0 $\pm$ 2.3 μ m) for both hybrid scaffolds. NTM5 cells exhibited a significant increase in metabolic activity following 14 days of culture for both scaffold types when compared to cells grown on 2D glass controls (p<0.0001). SEM imaging revealed NTM5 cells attached and elongated along the synthetic fibres, as observed in TM's natural environment. X-CT scanning revealed penetration of cells within the PLGA:PCL and PCL 20:12 scaffolds to be 28% and 89%, respectively.

DISCUSSION & CONCLUSIONS: By adjusting the materials used and electrospinning process, we have generated two scaffolds that mimic the hierarchical structure of the human TM. NTM5 cells cultured on these scaffolds successfully attached and infiltrated, and remained viable after 14 days in vitro. Functionality of these tissue-engineered scaffolds will be undertaken to determine the phenotype and behaviour of the TM cells as a potential cell therapy replacement.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: Eye, In vitro microenvironments

PP-094

Physiological mechanical testing and in-vivo mechanical behaviour to inform the production of biomimetic tissue engineered ligaments

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INTRODUCTION: Currently, anterior cruciate ligament (ACL) implants are assessed mainly by tensile tests. No studies have been performed to date to relate the full in-vivo hyper-elastic mechanical behaviour of the ACL in all ranges of motion with the design of tissue engineered ligaments. The aim of this work was to determine the electrospun structure most comparable to the in-vivo behaviour of the natural ligament during the execution of different dynamic activities.

METHODS: Biomechanics of 12 young participants were captured while they walk, run, cross-over cut, sidestep cut, jump vertically with both legs and with one leg. An OpenSim biomechanical knee model estimated the in-vivo ACL strain from the kinematic data [1]. In-vivo ACL tensile forces were determined with a well-established force/strain relationship [2]. 2D biomimetic prototype electrospun scaffolds were manufactured with 12% w/v of PVA dissolved in distilled water. 3D structures were created cutting the 2D electrospun meshes in rectangles of 2 x 15 cm and manually twisting them clockwise to form scaffolds of: one twisted filament, three twisted filaments and three twisted/braided filaments. 2D and 3D structures were crosslinked with 25% glutaraldehyde for 24 hours. 10-cycle tensile and shear tests with crosslinked and non-crosslinked samples were performed in dry and wet conditions (n=8 samples per condition). Mean and standard error of the mean were calculated for all conditions.

RESULTS: Three twisted/braided filaments scaffolds produced a maximum tensile stress of 38.0 ± 3.0 MPa in dry conditions, displaying properties most similar to the in-vitro mechanical behaviour of the natural ACL [3]. 1 twisted filament samples in dry and wet conditions bear the highest in-vivo maximum tensile stress for jumping [1]. Wet PVA scaffolds exhibited elastic behaviour with a well-defined toe region. Three twisted/braided crosslinked filament structure showed the highest maximum shear stress in dry conditions, allowing the samples to bear higher shear loads than with the other structures.

DISCUSSION & CONCLUSIONS: Three twisted/braided crosslinked filament structures mimicked the ACL tensile properties, showed a good resistance to shear loading and remained undegraded in phosphate-buffer saline solution. Further research will focus on determining shear force and torsion in the natural ACL to fully characterize its behaviour and manufacture ligaments that biomimic the properties.

ACKNOWLEDGEMENTS:

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Keywords: Nanomaterials (inc graphene), Knee

PP-095

Optimization of manufacturing parameters through machine learning techniques to create biomimetic vascular implants

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INTRODUCTION: Electrospinning is a manufacturing technique that creates a net of nano and microfibres able to mimic the natural extracellular matrix (ECM) of biological tissue. Electrospun scaffolds' morphology and mechanical behaviour can be tailored by modifying the environmental, solution and process parameters. This study aims to establish a manufacturing optimization methodology through Decision Trees (not previously investigated in electrospinning), novel Artificial Neural Networking models (ANN) and statistical approaches (multivariate analysis of variance, MANOVA / multivariate analysis of covariance MANCOVA) to produce biomimetic vascular implants.

METHODS: Polyvinyl alcohol-based scaffolds were manufactured with 10%, 12%, 14% and 16% w/v of PVA dissolved in distilled water and varying the values of flow rate (0.5-5 ml/h), voltage (18-25 kV), diameter of the needle (15-23G), distance between needle and collector (5-11 cm) and revolution of the mandrel (500-3000 rpm). Three novel models based on Chi-squared Automatic Interaction Detection (CHAID decision trees), Multilayer Perceptron (MLP) and MANOVA were used to simultaneously predict the diameter of the fibre and the inter-fibre separation. 2560 observations (diameter of the fibres and inter-fibre separation) were used to inform these morphological models. 60 observations informed a novel Multilayer Perceptron (MLP) and a MANCOVA model used to predict the mechanical properties (strain at break and ultimate tensile strength).

RESULTS: The prediction techniques led to an optimum set up of: 12% PVA, 1 ml/h flow rate, 20 kV, 8 cm between the needle, 18 G gauge needle and 2000 rpm. Optimised PVA scaffolds replicated the mechanical properties and morphology of the vascular tissue with an ultimate tensile strength of 6.17 ± 0.18 MPa, a strain at break of 97.39 ± 5.06 , fibre diameters of 126 ± 6.11 nm and inter-fibre separation of 1488 ± 91.99 nm. CHAID models predicted the morphology of the scaffolds with a standard error of 1.2%, ANN and MANOVA models showed comparable predictions with R² of 0.91, reducing the number of experimental trials.

DISCUSSION & CONCLUSIONS: CHAID models open an innovative and visual route to determine the optimum set up to manufacture scaffolds with comparable morphology and mechanical properties as the native tissue. Two-outputs ANN, MANOVA and MANCOVA models obtained comparable predictions; however, ANN predicted models demonstrated to be the most powerful option for non-parametric conditions. This work revealed for the first time that vascular substitutes with analogous biomechanical behaviour and physical structure to the native vascular tissue can be successfully achieved through CHAID, ANN and MANOVA/MANCOVA models.

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REFERENCES:

Keywords: Biofabrication, Nanomaterials (inc graphene)

PP-096

Stem Cell-Derived Secretome and its regenerative potential in tissue engineering protocols

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INTRODUCTION: Advances in tissue engineering during the past years have been focused on the rapid manufacturing and easy scale-up process of bioengineered tissues such as the artificial skin, oral mucosa and cornea. However, one of the main limitations on tissue engineering protocols is the requirement of high amounts of viable human cells in a short period of time (1). In this regard, human mesenchymal stem cells (MSC) are able to synthesize numerous trophic and inductive factors contained in their secretome, which could improve cell culture protocols and accelerate the generation of bioengineered tissues.

METHODS: In this study, we have analyzed the effect of three different types of secretomes obtained from MSC: adipose derived stem cells secretome (ADSC-s), dental pulp stem cells secretome (DPSC-s) and Wharton's Jelly stem cells secretome (WJSC-s) on human cultured cells. Human epithelial cells were isolated and cultured in basal medium and in medium enriched with MSC secretome for 120 hours. WST-1 cell proliferation tests were used to evaluate cell growth, and the number of cells at each follow-up point was assessed by flow cytometry. In addition, a preliminary analysis was performed in laboratory animals after 4 weeks of in vivo follow-up.

RESULTS: Our results revealed that the use of secretome derived from MSC was able to increase cell proliferation as determined by WST-1 after 24, 48, 72 and 120h of follow-up, with statistical differences with controls, especially when the DPSC and WJSC secretomes were used. Analysis by flow cytometry demonstrated an increment in the number of cultured epithelial cells, especially after 120h, with the highest efficiency found for DPSC secretome. In addition, in vivo analyses suggest that the use of the three types of secretomes is safe, as no side effects were detected in laboratory animals.

DISCUSSION & CONCLUSIONS: Our findings suggest that MSC-derived secretome can be safely used in tissue engineering protocols and could be consider as a promising alternative culture method to generate epithelial cell cultures of human skin, oral mucosa and cornea epithelial cells for use in tissue engineering protocols.

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Keywords: Biofabrication, Other

PP-097

BIOCLEFT: a regenerative approach to cleft palate treatment

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INTRODUCTION: The clinical management of children affected by cleft palate is very complex. In general, the surgical treatment is based on the transposition of palate mucosa grafts to close the midline defect (1). However, this approach is normally associated with severe growth alterations and hypoplasia of facial and palatal bones as a consequence of bone denudation secondary to the graft technique. In this work, we have developed a bioengineered palate mucosa for future use as an advanced therapy in children with cleft palate.

METHODS: A bioengineered model of the palate mucosa (BIOCLEFT) was fabricated in the laboratory from small biopsies of palate mucosa. Biopsies were digested enzymatically, and epithelial and stromal cells were cultured using specific culture media. Then, stromal cells were immersed within fibrin-agarose biomaterials, and a stratified epithelial layer was then generated on the surface of these stromal substitutes. Bioartificial palate mucosae were evaluated ex vivo and in vivo in newborn rabbits with a palatal defect to determine their potential usefulness to prevent the bone alterations found after the surgical repair of cleft palate.

RESULTS: Animals grafted with the bioengineered palate mucosa showed a more harmonic facial growth, with symmetric facial development. In contrast, control animals showed facial hypoplasia as a consequence of the surgical repair of the palatal defect. Differences between both groups were statistically significant for both the bone and the mucosal growth and development after 6 months of follow-up ($p=0.0004$). These results allowed us to design the BIOCLEFT clinical trial to evaluate the bioartificial palate mucosa in children. Preliminary results confirm the possibility to generate the BIOCLEFT model as an advanced therapy medicinal product in the laboratory.

DISCUSSION & CONCLUSIONS: The positive preclinical results obtained in laboratory animals suggest that the BIOCLEFT technology could contribute to optimize the current management of patients subjected to surgical repair of cleft palate defects. Final results of the clinical trial should determine the real utility of BIOCLEFT.

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Keywords: Translation and commercialisation (inc. clinical trials and regulatory approval), Other

PP-098

Towards cocultures with photosynthetically active microalgae: the effect of light on mammalian cells

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INTRODUCTION: The co-cultivation of mammalian cells with microalgae is an innovative concept to overcome one of the major limitations in tissue engineering and regenerative medicine, the shortage of oxygen.[1,2] Under illumination, photosynthetically active microalgae convert carbon dioxide into organic carbon compounds while producing oxygen. Thus, in such co-cultures, mammalian cells are exposed to light, which, for most cell types, does not reflect their natural conditions. In this study, we investigated the effect of white, red and blue light on viability and function of different mammalian cell types with the aim to identify an illumination regime that is not harmful to these cells.

METHODS: Cultivation under illumination with white (400-750 nm), red (600-650 nm) and blue (440-485 nm) light was conducted in a CO₂-incubator equipped with an RGBW LED strip. Human Dental Pulp Stem Cells (DPSC) and the murine Insulin-producing cell line INS-1 were seeded in tissue culture plates and continuously illuminated over 7 days from the bottom; control cells were cultivated in the dark. Moreover, INS-1 and murine Islets of Langerhans were encapsulated in alginate/methylcellulose hydrogels by 3D-bioprinting[3] and cultivated under white and red light vs. darkness. Cells were analyzed by live/dead staining, DNA quantification and ROS production; the functionality of INS-1 and islets was proven via glucose-stimulated insulin secretion.

RESULTS: In comparison to the dark control, illumination of all cell types with white light resulted in a significantly reduced viability accompanied by a strong reduction in the cell number – this was observed for 2D monolayer cultures of DPSC and INS-1 as well as INS-1 and islets within the 3D-bioprinted constructs. Similar observations were made for blue light illumination. In contrast, red light did not cause such adverse effects: viability and cell number as well as insulin secretion in response to glucose were comparable to the dark control. These findings were correlated with ROS production.

DISCUSSION & CONCLUSIONS: Advanced cell culture and tissue engineering concepts require more complex, artificial cultivation regimes. Long-term exposure of mammalian cells to light results in radiation energy input that is detrimental to viability and function at shorter but not at long wavelengths.

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Keywords: Diabetic healing, Innovation

PP-099

Biofabricated wet-spun core-shell hydrogel fibers for microvascular tissue engineering

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INTRODUCTION: Tissue engineering still lacks thorough vasculature, representing a major drawback in developing physiologically relevant tissue constructs. Hence, design and biofabrication of blood vessels at the microscale remain challenging. Also, fiber-based biofabrication techniques such as 3D bioprinting are considered time-consuming approaches in the field of microvascular tissue engineering. In this work, vessel-like 3D core-shell bundles have been rapidly fabricated using a novel wet-spinning (WS) system to reproduce the native architecture of the microvascular network.

METHODS: Two different hydrogel formulations were optimized. Fibrinogen- and alginate-based solutions were characterized. Rheological measurements on pre-polymer solutions and swelling test on scaffolds were performed to investigate the behavior of these biomaterials. SEM analysis was assessed to evaluate the microstructure of core-shell yarns. Afterwards, the fibrinogen-based formulation was loaded with a human umbilical vein endothelial cells (HUVECs)/mesenchymal stem cells (hMSCs) co-culture. Biomaterial inks were simultaneously extruded from a microfluidic co-axial nozzle immersed in a CaCl₂ coagulation bath to produce tissue-specific core and supporting tubular shell structures, respectively. Upon instantaneous gelation of alginate, fibers were collected to form packed fascicles. To stabilize bundles, secondary crosslinking was performed by immersing the yarns in thrombin solution for the enzymatic polymerization of the core. The engineered constructs were cultured up to 21 days. Both metabolic activity and cytotoxicity of encapsulated cells and constructs, respectively, were evaluated. Then, cell-laden scaffolds were investigated in terms of morphological characterization. Finally, immunocytochemistry will be performed to prove the formation of vessel-like structures.

RESULTS: The material characterization of the proposed formulations exhibited Newtonian-like behavior. Tissue-specific wet-spun core-shell fibers supported cell adhesion, migration, and alignment over the culture time, generating packed 3D cell-laden constructs that may recapitulate the microvascular network. Biocompatibility of the scaffolds was proven and proliferation assay confirmed consistent metabolic activity during the cell-culture period. In addition, scaffolds would likely reveal their endothelialization role, highlighting the potential of the two proposed bioinks in the frame of microvascular tissue engineering.

DISCUSSION & CONCLUSIONS: The proposed formulations can be used as non-shear thinning WS hydrogels to promote cell-specific proliferation. Fibers displayed metabolic activity and no cytotoxic response, validating the system for biofabricating soft hydrogel constructs. Encapsulated cells migrated within the hydrogel core towards the shell wall, aligning along the fiber axis direction to form a cellular layer. This study aims to highlight a new model promoting microvascular networks as a potential alternative to 3D bioprinted microvascularized constructs.

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Keywords: Biofabrication, Biomaterials

PP-100

Multiscale melt-electrowritten PCL constructs mimicking the architecture of native cartilage

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INTRODUCTION: Articular cartilage is a soft tissue that provides considerable dynamic load bearing capacity while reducing friction thanks to its unique composition and architecture. However, damaged cartilage is challenging to treat, mainly due to its avascular nature (Slater and Slater 2022). The development of viable articular cartilage grafts with appropriate biochemical and biomechanical properties is essential to provide successful treatment options in the future. Melt-electrowriting (MEW) has been recently established in the field of biofabrication and allows to produce polymeric fibrous constructs in a precise manner. MEW provides design freedom with respect to fibre orientation, spacing and dimension (Hrynevich, Elçi et al. 2018). The aim of this study was to fabricate a multi-scale construct consisting of a microfiber grid providing support, covered by a fine nanofiber-mesh mimicking the articular surface of cartilage.

METHODS: The multi-scale constructs were produced with an in-house developed MEW setup, with a microfiber grid using a parameter set established before (Santschi, Huber et al. 2022). To create the nanofiber-mesh, the polymer extrusion rate was reduced while the translational speed was increased, resulting in an increased mechanical stretch of the deposited fibre on the covering layer. The constructs were structurally assessed using scanning electron microscopy (SEM), mechanically under quasi-static conditions and tribologically using a pin-on-disc setup, to evaluate their potential as cartilage grafts.

RESULTS: The diameters of micro- and nanofibers were found to be $18.606 \pm 0.945 \mu\text{m}$ and $0.640 \pm 0.160 \mu\text{m}$ respectively. The MEW samples showed an average tensile E-Modulus of 9.40 MPa. The average coefficient of friction extracted across 3 repeats was 0.26 for a 0.13 MPa nominal contact pressure.

DISCUSSION & CONCLUSIONS: In this pilot study, it has been shown that multi-scale constructs with nanofibers are obtainable using MEW. In contrast to electrospinning, MEW allows for superior control of the nanofiber mesh and does not require the use of potentially toxic solvents. The tensile properties are in the range of cartilage tissue, the coefficient of friction however was found to be one magnitude above values reported in similar experiments involving articular cartilage. Although further development and analysis are required to fully evaluate the potential of MEW constructs mimicking the architecture of articular cartilage, this study provides a promising base for future research.

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REFERENCES:

Keywords: Additive manufacturing, Cartilage / joint and arthritic conditions

PP-101

Effect of alkaline treatment on PCL scaffold wettability: Impact of surface-to-volume ratio

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INTRODUCTION: Solution Electrospinning (SES) and Melt-Electrowriting (MEW) are established methods to fabricate fibrous polymeric constructs for tissue-engineering (Dalton, Woodfield et al. 2020). A well-studied polymer for MEW and SES is Poly(ϵ -caprolactone) (PCL). While being biocompatible and biodegradable, its hydrophobic nature hinders favorable cell interaction. This study aimed to evaluate the influence of the structure size of various PCL constructs on the required processing parameters of NaOH treatment, a well-established method to improve surface wettability (Bosworth, Hu et al. 2019).

METHODS: Nano- and microfibrinous PCL-membranes (nPCL and μ PCL) were manufactured using SES and MEW, respectively. The obtained membranes were immersed in NaOH solutions with concentrations of 0M (deionized water), 1M, 2M and 4M for a duration of 1, 2, or 4 hours (N=3 per condition). The impact of the NaOH treatment on the structures was evaluated structurally using scanning electron microscopy and mechanically by uniaxial tensile testing under quasistatic conditions. Most importantly, the surface wettability or water contact angle (WCA) was analyzed using a drop shape analyzer.

RESULTS: No structural alterations, such as fibre morphology, have been observed for NaOH treated nPCL and μ PCL. Untreated control nPCL and μ PCL revealed $WCA > 115^\circ$ for all immersion durations, indicating the superhydrophobic nature of PCL. Treating μ PCL with NaOH for one hour decreased the contact angle to $106^\circ \pm 8.00^\circ$ (1M), $57^\circ \pm 37.7^\circ$ (2M) and $62^\circ \pm 19.7^\circ$ (4M). A treatment time of two hours increased the effect slightly, while four hours treatment rendered the surfaces completely hydrophilic ($WCA = 0^\circ$) for all concentration of NaOH. Untreated nPCL showed similar contact angles above 119° for all immersion durations. However, all treated nPCL scaffolds showed complete hydrophilicity ($WCA = 0^\circ$). Mechanical testing revealed no significant difference of the tensile modulus (3.45 ± 1.26

MPa and 11.06 ± 0.46 MPa for nPCL and μ PCL respectively) for any treatment, however NaOH treated samples tend to break, indicating brittle behaviour.

DISCUSSION & CONCLUSIONS: The present study investigated the potential impact of structure dimension on the required NaOH treatment parameters. WCA analysis highlighted that nPCL is more sensitive to treatment time and concentration, compared to μ PCL. This is most likely attributed to nPCL's higher surface-to-volume ratio compared to μ PCL, therefore rendering the treatment more efficient. While no impact on the structure morphology and elastic behaviour was found, an increased brittleness was observed for NaOH treated samples especially μ PCL-membranes. This may be due to the higher impact of single fibre breakage, considering the lower fibre density and the consequently higher stress per μ PCL-membrane fibre.

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REFERENCES:

Keywords: Biofabrication, Polymers - natural / synthetic / responsive

PP-102

Tuning the location and concentration of surface-installed click type functional groups on additive manufactured constructs for osteochondral regeneration

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INTRODUCTION: Current clinical methods to treat osteochondral (OC) lesions are associated with limitations, such as fibrocartilage formation, and therefore tissue engineered constructs are being designed to restore tissue function. Additive manufacturing (AM) has gained a lot of attention as candidate for osteochondral construct development. When using thermoplastic polymers, we observe two challenges with the current AM OC constructs. (1) The OC tissue has predominantly a gradual transition from cartilage to bone, but current print heads only allow for the stratified designs. (2) There is limited work on creating a versatile platform with control over the surface density of biologically active groups. To this end, we set out to design an AM construct in which gradual control over human mesenchymal stromal cells (hMSCs) differentiation is allowed via surface exposed differentiation-inducing peptides. Additionally, we investigated a method to tune the peptide concentration on the surface.

METHODS: We synthesized low and high molecular weight (MW) PCL-derived polymers with clickable reactive groups. A novel in-house developed printhead was used to mix polymers in a gradual fashion into a 3D scaffold. To tune the surface density of the reactive groups, we blended low MW with high MW polymers. After thermopressing, we reacted fluorescent dyes on the surfaces for spectrophotometric evaluations. Finally, we assessed hMSC differentiation in-vitro using BMP-2 and TGF- β derived peptides.

RESULTS: We successfully mixed two PCL-derived polymers in a scaffold with control over the length and location of the gradient across the scaffold. Using polymeric blends, we showed an significant enhanced of the surface concentration of the reactive groups, from 100 to 400 nM in 2D. All these blends were still successfully printable. Using the high MW PCL, so with the lowest peptide concentration, we observed a limited or no effect on osteochondral differentiation of hMSCs. In the 2D proof-of-concept study using the blends, we observed an enhanced effect on osteogenic markers in relation to the enhanced surface density.

DISCUSSION & CONCLUSIONS: Here, we used a novel AM printhead to create next generation OC construct with continuous material gradients containing chemical handles. Due to use of orthogonal chemistry, virtually any peptide can be attached to the surface depending, making these scaffolds interesting for the broader community. Moreover, biological responses tend to be concentration dependent, and we unlocked a range of concentrations via a blending strategy.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Biomaterials

PP-103

3D printed foamed scaffolds for tissue engineering

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INTRODUCTION: The aim of this work is to present the results obtained by an innovative technology that combines the flexibility of the 3D printing process to design complex geometries and the simplicity of a physical foaming process to create structures with two separate levels of porosity: interstrand macroporosity obtained by printing layers-by-layers and the second one the intrastrand microporosity formed by the foaming process, to produce scaffolds for tissue engineering.

METHODS: The obtained structures were characterized by SEM analysis, apparent density measurements and microCT analysis.

RESULTS: The microCT revealed scaffolds have an open porosity of around 85%, a score which allows to potentially destine the structures to several applications in tissue engineering as reported in the literature. Furthermore, human dermal fibroblasts were seeded on scaffolds after sterilization and pre-conditioning in the culture medium to test biocompatibility. Results demonstrated the ability of the cells to adhere to the scaffolds and proliferate over time. Confocal images showed that the cells acquired their typical elongated morphology on scaffolds, similar to what showed by the SEM images. These images show also how the cell adhere and proliferate in the internal cavities present in the structures due to the foaming process.

DISCUSSION & CONCLUSIONS: Nowadays, less expensive technologies and less time-consuming alternative methods that cover a wide range of controlled pore sizes and morphology are highly demanded to enable full exploitation of the rich design space offered by hierarchical structures. And this method by tuning the physical voids, which has been obtained by controlling the processing parameters (namely, extrusion velocity, extrusion temperature, and absorption time), allows a high level of customisation of the final structure. Due to the first promising results of cell culture, it can be considered a favourable technology with application in the field of tissue engineering and regenerative medicine.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: 3D printing and bioprinting,

PP-104

Microcarriers Fabricated by Two-Photon Lithography for Expansion of Mesenchymal Stem Cells

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INTRODUCTION: Mesenchymal stem cells (MSCs) can differentiate into a range of cell types for tissue regeneration but for effective treatment large numbers of cells are needed due to low retention in defect sites. Expansion of MSC populations to meet clinically relevant levels is often achieved through culture on commercially available microcarriers which are predominantly smooth polystyrene microparticles.

Complex architectures exhibiting specific biophysical cues can increase MSC expansion capacity and control differentiation [1]. The production of bio-instructive microcarriers using biophysical cues have the potential for enhanced cell expansion that surpasses currently available technologies as well as the capability to control differentiation.

METHODS: Two photon lithography (2PL) can create highly accurate microscale structures with feature sizes of 200nm. This technology was employed to create microcarriers with varying architectural designs. Novel methodology for directing cells to geometries was created and utilised for MSC cell expansion with analysis via macroconfocal microscopy and DNA quantification. Analysis of the morphological properties of cells upon these architectures as well as their expansion proficiency was assessed. Scale-up and free-floating culture of MSCs allowed MSC phenotypic analysis.

RESULTS: A variety of produced designs exhibited increase in cell proliferation and interaction with structure features. Morphological analysis of cells and DNA quantification determined microcarrier designs that lead to a significant increase in expansion yield in comparison to a conventional spherical design. Best expansion yields were seen in Buckminsterfullerene styled structures with hollow interiors and porous outer shells, this effect was maintained when correcting for changes in surface area between designs. Analysis of stem cell markers across the expansion period indicated maintenance of the stem cell phenotype over the course of the expansion.

DISCUSSION & CONCLUSIONS: It was noted from early screening experiments that different architectures exhibited varying levels of cellular preference for certain geometrical feature types and sizes. With these results normalised to surface area and seeding efficiency for each design, it is believed that increased expansion is attributed to the biophysical stimuli of the geometry. This study demonstrates novel 2PP fabrication techniques to assist cell culture upon samples and has identified several architectures that interface with MSCs at the mechanobiological level to enhance proliferation. This work aims towards producing novel microcarriers for in-vitro expansion but due to the bioinstructive properties may hold value in the form of in vivo particle based scaffolds.

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Keywords: Additive manufacturing, Biomechanics / biophysical stimuli and mechanotransduction

PP-105

Additive manufactured polyether-ether-ketone implants for orthopaedic applications and the promotion of integration of bone and soft tissue

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INTRODUCTION: Polyether-ether-ketone (PEEK) is believed to be the next-generation biomedical material for orthopaedic implants that may replace metal materials because of its good biocompatibility, appropriate mechanical properties and radiolucency. Currently, PEEK implant represented by intervertebral fusion cage has been used successfully for many years. Till now, there is no customised PEEK orthopaedic implant made by additive manufacturing licensed for the market, although clinical trials have been increasingly reported. The poor integration between Poly-ether-ether-ketone (PEEK) with hard and soft tissue represents a major challenge of PEEK orthopaedic implant owing to its chemical inertness.

METHODS: Here we investigated the influence of hydroxyapatite (HA) contents and pore size of additive manufactured (AM) HA/PEEK composites scaffolds with HA content of 0, 20 wt% and 40 wt% on the integration with bone and soft tissues through cellular experiments and animal experiments. The composite scaffolds were manufactured by fused filament fabrication.

RESULTS: The cell experiment showed that the adhesion, proliferation, osteogenic differentiation and mineralization ability of mesenchymal stem cells (BMSCs) as well as the proliferation and adhesion of myofibroblasts on the PEEK/HA scaffolds were significantly improved. The in-vivo experiment demonstrated significant higher bone ingrowth and tighter adhesion of soft tissue in the HA/PEEK scaffolds. The ingrowth volume (24.3%) of 40 wt% HA/PEEK scaffold after implantation for 12 weeks was comparable to the bone ingrowth reported for metallic or ceramic scaffolds. The bonding strength between HA/PEEK scaffolds and soft tissue was dominated by the geometry dimension, while HA has a facilitative effect on the tight adhesion of the soft tissue to the PEEK-based composite scaffold, which is vital for avoiding postoperative effusion.

DISCUSSION & CONCLUSIONS: The present study provides engineering-accessible design principles on material components and geometry of AM PEEK-based composites orthopaedic implant for improving the integration with bone and soft tissue. Design criteria, including geometric matching, functional restoration, strength safety, early fixation, long-term stability and manufacturing capability, were summarised based on the understanding of the integration of HA/PEEK composites with natural tissues. Typical clinical applications of PEEK-based 3D printed implant was carried out according to the design criteria.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

PP-106

Computational Modelling and Topology Optimisation of Additive Manufactured Bone Plates for Long Bone Fractures

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INTRODUCTION: Large bone defects caused by trauma or disease remain as one of the major causes of disability worldwide, often requiring surgical intervention to facilitate the healing process. For that purpose, inert metals such as titanium or stainless steel are commonly used for the fabrication of internal fixation plates capable of stabilizing the fracture and guiding bone ingrowth. However, the clinical application of these devices is not without shortcomings and long-term complications are often reported including the need for revision surgeries, infections, radiological artefacts and inadequate bone regeneration due to stress shielding effects. The latter arises due to the stiffness mismatch between the metallic plate and the native bone resulting in little to no mechanical stimulation of the tissue – a key aspect in extracellular matrix production by osteoblasts. Here we combine reverse engineering, computational modelling tools and Additive Manufacturing (AM) techniques to develop topologically optimized plates with tailored physical properties for enhanced osteogenic regeneration and skeletal integration.

METHODS: A three-dimensional (3D) computer aided design (CAD) model of the plate was generated by laser scanning (3D Shining, China) and reverse engineering a commercially available eight-holed locking compression plate (Depuy Synthes, USA). The plate was subsequently assembled into a construct with simplified humeral geometry and fixed with six screws and imported into Abaqus CAE (Dassault Systems, France). The humeral shaft was fractured in accordance with three frequently seen cases; traverse, oblique, and a wedged shaped segmental fracture, often referred to as a butterfly fracture. An initial loading case of 600N axially at the proximal head, fixed at the distal end under encaster boundary conditions.

RESULTS: Initial results showed the predominant stresses are seen in the centre of the plate, with von Mises stress increasing by a factor of 10 from transverse to oblique, to butterfly, with a maximum principal stress of 1.38GPa.

DISCUSSION & CONCLUSIONS: Based on obtained results and targeted strain energy levels, material was removed in increments of 10% by volume and thickness reduction up to 50%. This allowed for a substantial reduction in the thickness of the plate whilst minimising unwanted stress shielding effects. The latter was confirmed by evaluating the resulting stresses in the bone construct using Abaqus CAE software and 4-point-bending method, as per ASTM standards. The next step will involve 3D printing of the metallic plates and validate the computational modelling results experimentally using synthetic bone.

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Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)

PP-107

Textile based electrospun PCL-PLA core-sheath nanofiber yarn scaffolds for tubular tissue applications

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INTRODUCTION: Cardiovascular and musculoskeletal tubular tissues such as aorta, intestine, trachea and esophagus carry vital functions of the body. Due to numerous diseases hollow organs are of great interest for tissue engineering but there is little emphasis on the development of scaled, 3D structures, which are more clinically relevant.

Hollow tubular organs generally have a similar structure, consisting of three multicellular distinct tissue layers, and are characterised by complex mechanical behaviour¹. Textile technologies can create seamless 3D structures that mimic anisotropic and hierarchical properties of these tissues. Nanostructured textile scaffolds made of nanofibrous yarn provide high surface area, controllable porosity and favourable environment for cell attachment. Electrospinning is commonly used to produce fibres in nano-scale from natural or synthetic polymers, which can closely mimic the fibrous part of the extracellular matrix. Nanofibrous yarns can be developed into complex textile designs for biomedical applications².

METHODS: In this project, a conjugated electrospinning setup was used to develop a PCL-PLA core-sheath nanofibre-coated yarn for the fabrication of textile structures for tissue engineering applications. Woven and knitted structures were prepared with the nanofibrous core-sheath yarn. Scanning electron microscopy (SEM) was used to characterise the nanofibre-coated yarn. LIVE/DEAD and Alamar blue assays were used to examine the effect of the fibres on NIH3T3 (mouse embryonic fibroblast cell line) and human umbilical vein endothelial cells (HUVECs).

RESULTS: SEM demonstrated that relatively uniform and bead-free fibres with smooth surfaces were obtained. LIVE/DEAD and Alamar Blue assays demonstrated that the resultant scaffolds were biocompatible and allowed cell growth. Textile scaffold mechanical properties will also be presented.

DISCUSSION & CONCLUSIONS: The results demonstrate the potential of textile-based scaffolds as a versatile and scalable approach for tissue engineering. Advanced electrospinning produced long-length nanofibrous yarn with appropriate mechanical and biological properties made of a PCL-PLA composite. The mechanical and biological assessments will be provided. This study contributes to the development and design of hybrid PCL/PLA nanofiber scaffolds for soft tissue engineering research and applications.

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Keywords: Biomaterials,

PP-108

Studying cellular integration within tubular electrospun scaffolds

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INTRODUCTION: One of the key factors in fabrication of a tissue engineering construct is having cellular infiltration in all regions of the scaffolds. Having densely packed fibrillar structure, electrospun scaffolds usually provide a spatial culture pattern limited the few micrometres of the surface [1]. Here, we aim to fabricate thick fibrillar scaffolds capable of cellular infiltration using electrospinning.

METHODS: Different electrospun scaffolds were made using 12% Polycaprolactone (PCL) solution (Mw=80K) either in chloroform or Chloroform/DMF (19:1). All samples were made using 1cm diameter collecting drum, rotating at 200 rpm, placed at 25 cm distance from a gauge 18 blunt needle. Different applied voltage and flow rate were used for fabrication of different samples. Scaffolds were characterized using field emission scanning electron microscope (FE-SEM) and universal tensile testing machine for their structure and mechanical behaviour. Then, samples were cultured with fibroblasts for a duration of 7 days. Cellular migration and arrangement of cytoskeleton were followed up after 1, 3, and 7 days of culture using FE-SEM and confocal microscopy.

RESULTS: We were able to create electrospun mats at different voltage and flow rates. FE-SEM images have shown a bead-less and homogenous structure, with micro-range thickness. Addition of DMF to chloroform have enhanced the spinnability but reduced the diameter of fibres. Pore sizes across different samples were comparable to the size of a single cell. FE-SEM and Immunohistochemistry images have shown the migration and uniform arrangement of cells through the depth of the electrospun scaffolds.

DISCUSSION & CONCLUSIONS: Electrospinning provides a versatile and cost-efficient method in fabrication of tissue engineering scaffolds. However, small pore size limits the infiltration of cells in the depth of electrospun scaffolds, makes them not ideal for fabrication of 3D tissue engineering constructs. Here, we have shown that using high fibre diameter scaffolds, we can have large pore sizes which allows infiltration of cells in depth of scaffolds. These structures can be ideal for further use together with other methods including 3D bioprinting of hydrogels to create stand-alone structure with lower degradation time, which allows long-term culture in-vitro, and render handleability for placement of the implant in vivo.

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Keywords: Fibre technology, Biomaterials

PP-109

A Novel High Porous PCL Wet-electrospun Bionic Matrix Mimic Cartilage Renew in Vitro

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INTRODUCTION: Cartilage zonal structures perform discrete function [1]. The cartilage ECM on the superficial zone show oriented alignment and is regarded as the renewal area. Chondrocyte progenitor cells proliferate and differentiate horizontally from the superficial zone to the deeper zone. Thus, the idea that a critical size defect at the cartilage superficial zone disrupts the cartilage renewal process and promotes the vicious cycle of osteoarthritis, gets more attention.

In this study, we developed a novel high porosity matrix with oriented fibrous structure, which mimics both the cartilage superficial ECM structure and the renewal process of the cartilage which could be applied in cartilage tissue engineering.

METHODS: A high porosity oriented matrix was fabricated via an improved dynamic wet-electrospun which drove liquid via magnetic stir bar, creating vortex where a polystyrene (PS) sphere floated, rotated and entangled with PCL electrospun fibre. PCL matrix was collected, frozen, cut and freeze-dried into disks. The porosity was tested via the gravity method. The morphology was observed via SEM. Cell-seeded scaffolds were used to research cell-matrix interfacial via SEM and cell proliferation and migration via cryosection and DAPI staining.

RESULTS: The measurement of porosity revealed a $99.5 \pm 0.1\%$ porosity, followed by the SEM observation, showing an oriented yarn-based structure (Average fibre diameter: $1.48 \pm 0.62 \mu\text{m}$) with large pores between the yarn and yarn. This highly porous matrix formation could be attributed to the interface between ethanol and polymer solution jet and its viscoelastic properties.

56.02% of cells were attached to scaffolds after seeding, compared with tissue culture plate group. Healthy, spread cells attached on yarns were observed via SEM after 24h culture. The cell number increased at all timepoints. Further, two timepoints, the D1 and D14 were cryosectioned and stained with the DAPI and found that the cell covered the superficial area and then migrates and infiltrated to the deeper zone of the matrix, which mimics the renewal process of cartilage.

DISCUSSION & CONCLUSIONS: An improvised wet-electrospinning set-up has been developed to fabricate high porosity oriented PCL matrix. The study demonstrated the resultant PCL Matrix has a porosity of 99.5% which allow cell to migration. The oriented structure mimics the cartilage ECM. The histology study indicated the reproduction of the cartilage renewal process in vitro.

ACKNOWLEDGEMENTS:

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Keywords: Stem cells - general, Fibre technology

PP-110

core-shell rifampicin-loaded electrospun polycaprolactone membranes coated with silver nanoparticles for tissue engineering applications

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INTRODUCTION: A broad variety of Electrospun polycaprolactone (PCL)-based matrices has been developed for a various range of tissue engineering applications such as Guided bone regeneration (GBR), ureter regeneration and wound dressing; thank to their ability to mimic the architecture of extracellular matrix (ECM), slow biodegradability, and biocompatibility. In this work, exploiting PCL versatility and easy of process, electrospun core-shell nanofibers were developed. To discourage the bacteria infections, the scaffolds were double-functionalized with antibacterial compounds, adding the antibiotic rifampicin in the core and silver nanoparticles on the shell of the co-axial fibers.

METHODS: Electrospun PCL and PCL/Rif planar co-axial fibers were obtained by including the antibiotic in the core. Matrix hydrophilicity was improved by air-plasma treatment and verified by wettability measurements. The core-shell structure was confirmed by TEM analysis and the release of Rif from fiber core was analyzed by UV spectrophotometry. Afterwards, the in-situ synthesis of nAg on the shell was performed by sequential coating with dopamine hydrochloride and silver nitrate and characterized through SEM-EDS analysis. To investigate the antibacterial efficacy of electrospun membranes, *E. coli* and *S. aureus* were considered. Moreover, their biocompatibility was assessed on urothelial cells (UCs), murine fibroblasts (NIH-3T3), and human osteosarcoma cells (MG-63).

RESULTS: The PCL and PCL/RIF co-axial membranes showed thick, homogeneous, and defect-free fibers with a typical core-shell morphology and suitable water wettability already after a 30-seconds plasma treatment. The Rif inclusion in the core ensured its protection from the plasma treatment, with a comparable release for up to 1 week between the untreated and treated mats. Meanwhile, the nAg revealed a homogeneous distribution on the fiber shell and the double core-shell functionalization allowed a combined inhibitory effect against *S. aureus* (Rif) and *E. coli* (nAg) without affecting membrane biocompatibility towards UCs, NIH-3T3, and MG-63.

DISCUSSION & CONCLUSIONS: Electrospun polycaprolactone-based co-axial membranes with antibacterial properties were produced with the aim to develop a biomaterial suitable for tissue engineering applications such as skin, bone, and ureter regeneration. In details, the membranes showed an architecture capable to mimic the ECM and possess suitable biological and antibacterial properties for the proposed applications. Therefore, thanks to the possibilities offered by the electrospinning, such as the specific tailoring of the membranes, the material here proposed is promising for several tissue engineering applications.

ACKNOWLEDGEMENTS:

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Keywords: Drug delivery, Biomaterials

PP-111

New 3D models of breast tissue to understand the role of stiffness and physico-chemical properties on breast cancer cell stemness

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INTRODUCTION: Within the tumour microenvironment, several physico-chemical properties such as extracellular matrix (ECM) stiffness, extracellular pH and interstitial fluid flow are dysregulated during tumour progression. Traditional cell culture and animal models, although being a valid alternative to study variations of tissues properties, do not provide sufficient control to further study cause-effect relationships between tumour microenvironment and cancer cells. Particularly, in breast cancer tissue stiffness is known to regulate tumour progression. Breast cancer stem cells (B-CSCs), a dynamic population within the tumour milieu, is a key player in breast tumour progression, metastasis and therapeutic resistance. While some studies explore effect of biochemical factors present within the TME on B-CSC population, we firstly herein report on the direct effect of ECM properties on B-CSC population [1].

METHODS: Alginate-based hydrogels were designed to encapsulate humans breast cancer cells (i.e. MCF-7, MDA-MB 231) and to model the breast tissue at different stages of cancer progression. Model parameters to match breast tumour pathophysiology were defined as hydrogel compressive moduli (2-10 kPa), composition and density (gelatin content), extracellular pH (6.5, 7.4) and perfusion (500 μ L/min). We investigated how these parameters impacted on human breast cancer cell viability and proliferation, morphology, markers expression for B-CSC population (ALDH, CD24, CD44, E-cadherin and vimentin).

RESULTS: The new 3D in vitro models enabled a fine control of the breast tumour microenvironment to monitor cell response for up to 2 weeks. In vitro phenotypes observed were found similar to in vivo observations, with increased proliferation in softer hydrogels and physiological pH (7.4) in MCF-7 and increased irregularity in cell clusters formation with increased gelatin in MDA-MB-231. Moreover, stiff hydrogels (10 kPa) and low extracellular pH (6.5) are the two parameters identified to mostly impact on B-CSC dynamics, showcasing increased population of both mesenchymal and epithelial CSCs in the cell lines tested. This suggests a direct impact of the physical microenvironment on B-CSC population and the metastatic potential of breast tumour.

DISCUSSION & CONCLUSIONS: The proposed models can be used to delineate and understand better interactions between ECM and cells, offering a new tool to control ECM physical parameters. Engineering the tumour microenvironment could be further used as testing platforms for effective identification of specific phenotypes as function of relevant tumour physico-chemical properties.

ACKNOWLEDGEMENTS:

REFERENCES: [1] DOI: 10.1016/j.actbio.2022.08.074

Keywords: Cancer, Microenvironment and niche engineering

PP-112

A 3D Organotypic "ex vivo" Bone Marrow Slice Culture Model to Investigate Cell Responses to Bacterial Infection

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INTRODUCTION: Infections of the bone marrow by "*Staphylococcus aureus*" ("*S. aureus*") species, one of the main colonisers in prosthetic joint infections and osteomyelitis, are often associated with increases in inflammation and influx of pro-inflammatory cytokines leading to osteonecrosis and bone loss. It is at these damaged necrotising areas that bacterial species colonising an implant invade into the bone marrow cavity. The pathologic mechanisms leading to bone loss are poorly understood with limited reproducible model systems available that can be carefully manipulated to improve understanding. The aim of this study was to develop an ex vivo osseous model that provides a 3D organotypic culture environment promoting viability of cell populations and to validate the responsiveness of this system to bacterial virulence following infection with "*S. aureus*" species.

METHODS: Femoral slices of 1.5mm thickness were prepared from 28 day old male Wistar rats, embedded in a semi-solid agar based medium and cultured at the liquid-air interface for 48hr in α MEM, supplemented with 30ng/mL RANKL and 20ng/mL M-CSF, along with a microinjection of mixed bone marrow cells. Maintenance of cell populations was confirmed by digital histomorphometry and immunocytochemistry identifying CD105, CD14, CD68 and neutrophil elastase as markers for mesenchymal stromal cells (MSCs), monocytes, macrophages and neutrophils respectively. Femoral slices were also cultured for 24h in the presence of agar inoculated with two different "*S. aureus*" strains (Oxford reference and osteomyelitis isolate 7791). Following infection, tissue slices were examined by digital histomorphometry and immunocytochemistry for apoptosis markers and necrosis LDH activity. Secretion of pro-inflammatory cytokines, IL-1 β , IL-6, TNF α was assessed by ELISA. Heat inactivated bacteria provided negative controls.

RESULTS: Maintenance of cell types following culture were observed with immunocytochemistry. Histological examination of tissue slices following infection with either strain demonstrated attachment of bacteria to the extracellular matrix of cultured tissue. Cell viability analysis following immunocytochemistry suggested apoptosis of cells following culture with bacteria and LDH analysis suggested increased evidence of necrosis by 19hrs in culture with viable bacteria compared to controls. Cells within cultured tissue slices secreted pro-inflammatory cytokines TNF α and IL-1 β with secretion of cytokines significantly higher in tissue infected with strain 7791 ($p < 0.005$). IL-6 secretion by cells in both infected tissue slices and controls was low.

DISCUSSION & CONCLUSIONS: This data suggests potential successful development of an ex vivo osseous slice culture model capable of supporting heterogeneous cell populations. Infection of the culture model demonstrates an immune response and secretion of pro-inflammatory cytokines representative of the clinical environment

Keywords: Bone and bone disorders (osteoporosis etc), Disease models

PP-113

Lumen-based microfluidics for cancer-on-chip devices

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INTRODUCTION: Most breast cancer related deaths are not caused directly by primary tumor, but by secondary tumors formed through metastasis to other organs [1]. Metastasis is a complex cascade that we still poorly understand due to the limitations of current in-vitro models. Hence, we focus on modeling cancer metastasis on a chip, via introducing the relevant physiological factors in the tumor microenvironment. To obtain such a model, we exploit our 3D sugar-printing technology to create luminal channels with circular cross section to mimic (micro) vasculature and breast duct in ductal carcinoma.

METHODS: We use 3D sugar-printing technology [2] to create sugar glass structures that subsequently form perfusable lumens. These sugar glass structures can be cast either in Extra Cellular Matrix (ECM), or synthetic polymers; after the ECM material or synthetic polymer is cross-linked and the sugar glass is selectively dissolved, hollow perfusable lumens are obtained. When the lumens are seeded with endothelial cells, they form the (micro) vasculature. Combined with either a neighboring lumen for cancer cell culture, the process of cancer invasion, migration through ECM, and intravasation can be studied. Furthermore, neighboring lumens can be filled with different types of hydrogels or cell types to mimic the pre-invasive and invasive cancer microenvironment.

RESULTS: We have developed a vessel and duct with 3D luminal geometry where cell-cell tight junctions are present, as a basis for further study to recapitulate metastatic cascade.

DISCUSSION & CONCLUSIONS: Cancer-on-Chip devices enable us to mimic key biophysical characteristics of the breast cancer microenvironment. We use 3D sugar printing to create lumens directly in the ECM which enables us to study the invasion and subsequent intravasation. This way, we avoid using synthetic materials which often have drawbacks for cellular experiments. However, casting lumens or other circular structures in synthetic polymers, is still a feasible option. Lumen channels with round cross section provide more biophysical-alike perfusion. ECM structure and properties are expected to play a role in breast cancer invasion, and our model composed of multiple cell types and different concentrations of hydrogels can potentially lead to better understanding of pre-invasive and invasive breast cancer. Our model has also the capability to add more components of the in vivo cancer microenvironment, such as immune cells or oxygen gradients.

ACKNOWLEDGEMENTS:

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Keywords: 3D printing and bioprinting, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-114

PDAC-on-chip: modeling the stromal and pancreatic cancer cells crosstalk in vitro

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INTRODUCTION: The pancreatic cancer is the most frequent type of exocrine pancreas tumor and one of the leading causes of cancer-related death worldwide with a five-year survival rate of below 9% [1]. Current research is focusing on better understanding pathologies onsets to tailor effective treatments through the development of pancreatic cancer in vitro models. To date, the tumor-stroma crosstalk remains extremely challenging to be reproduced and monitored in functionally effective models. Therefore, the main purpose of this work is to design and develop an innovative biomimetic 3D in vitro model that replicates the composition of pancreatic exocrine unit in a microfluidic device.

METHODS: A multilayer PDAC-on-chip composed by a top and a bottom layer was developed to culture the PDAC cells and the pancreatic stellate cells (PSCs) embedded in a type I collagen gel in the top and bottom layers, respectively. The presence of a biomimetic nanofibrous membrane in the middle of the chip permits to control the interactions between the two cell lines and to easily analyze the effect of the crosstalk on cell behavior.

RESULTS: PDAC-on-chip system was validated by demonstrating that human fibroblasts seeded in a 3D collagen matrix in the bottom microchannel also change to a myofibroblasts-like shape with increased expression of α -SMA and secrete higher amount of IL-6 cytokines. Bortezomib and Gemcitabine were used as model drugs to test the drug efficacy to induce HPDE-KRAS cell death using PDAC-on-chip.

DISCUSSION & CONCLUSIONS: The developed PDAC-on-chip chip design resembles the acino-ductal unit of the exocrine pancreas composed by acinar and ductal epithelial cells surrounded by PSCs which is the anatomical unit where the PDAC mainly develops. Finally, this microfluidic system serves as a powerful tool to mimic evolution steps of PDAC (e.g. fibroblasts activation) and resulted suitable for the evaluation of drugs efficacy.

ACKNOWLEDGEMENTS:

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In vitro microenvironments

PP-115

A novel organ-on-chip-based in vitro approach for co-culturing 3D human cancer tissues and circulating capillary flow-driven immune cells for more predictive drug testing and human disease modeling

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INTRODUCTION: The human disease modeling for basic research and drug testing purposes is currently carried out through 2D cell culture in static conditions, and in vivo xenografts or genetically engineered animal models, but predictability, reliability, and complete immune compatibility remain important challenges. For this aim, novel 3D, fully humanized in vitro cancer tissue models have been recently optimized by adopting emerging technologies such as microphysiological systems (MPS) and 3D cell laden hydrogels. In particular, a novel Multi-In Vitro Organ (MIVO) MPS platform has been recently adopted to culture 3D clinically relevant size cancer tissues under proper physiological culture conditions to investigate the anticancer treatments efficacy.

METHODS: Biologically relevant cancer samples (up to 5 mm), have been developed by using a alginate base structure, resembling the extracellular matrix. The stiffness of such gels has been optimized to support a in vivo like tumor cells viability, cluster formation and migration. Ovarian and neuroblastoma cell laden hydrogels (1 million cells/ml) have been cultured within the MIVO chamber, while either testing molecules (cisplatin) or human immune cells (Natural Killer cells, NK) respectively circulate in the MPS mimicking the blood capillary flow. The tumor cell proliferation and viability were investigated in such dynamic cell culture conditions to assess the cytotoxic efficacy of the treatment. When the systemic administration of cisplatin was simulated within the MPS, the anticancer drug efficacy was also tested and compared to the animal model. When NK cells were placed in circulation, their extravasation through a permeable barrier resembling the vascular barrier, and infiltration within the neuroblastoma cancer tissue were analyzed.

RESULTS: A human 3D ovarian model was developed and treated with Cisplatin in static conditions, within MIVO, and in the xenograft model. Similar tumor regression was observed in MIVO and in mice, while the static culture displayed an unpredictable chemoresistance, due to unreliable drug diffusion within the 3D matrix. A human 3D neuroblastoma cancer model with proper immunophenotype was optimized to develop a complex tumor/immune cell coculture as a paradigm of an immune-oncology screening platform. Importantly, a tumor-specific NK cell extravasation was observed under dynamic culture, with NK cells able to infiltration within the 3D tumor where they induced cancer cells apoptosis.

DISCUSSION & CONCLUSIONS: We generated a relevant human disease model, through the adoption of a MPS system, that can be efficiently employed as a drug screening platform but also for better investigating crosstalk among immune /tumor cells.

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Keywords: In vitro microenvironments, Enabling technologies

PP-116

TTOP perfusion system: a modular dynamic MicroPhysiological device for compartmentalized, continuously perfused and optically accessible cultures of tissue barriers

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INTRODUCTION: Conventional static “open-well” in vitro barrier models aim to replicate the compartmentalization found in vivo, by culturing cells on porous substrates, that separates the apical and basolateral compartments[1]. However, they cannot provide fluid flows to mimic the perfused in vivo environment. Micro Physiological Systems (MPSs) allow to dynamically stimulate in vitro tissue barriers, however their intrinsic “closed-well” design limits the accessibility to the biological sample, introducing complex procedures[2]. For these reasons we developed True Tissue On Platform (TTOP), a modular and versatile platform, composed by an “open-well” static preparation module and a “closed-well” perfusion module, aiming at integrating simple preparation procedures and advanced dynamic conditions.

METHODS: A laser micromachined cartridge was positioned in the “open-well” static module compatible with standard 12-well plates, enabling the preparation of an intestinal in vitro model on a microporous membrane. Caco-2 cells were cultured until confluence. Then, the “open-well” static module was placed in the “closed-well” perfusion module, enabling continuous recirculation of apical and basolateral culture media generated by embedded piezoelectric micropumps (from 100 to 1200 ul/min). The perfusion module integrates bubble traps, to capture flowing microbubbles and reservoirs, to easily replace the culture medium. Flow rates were programmed through an Arduino-based control unit and a touch screen. Finally, we retrieved the cartridge with a simple procedure and performed fluorescence microscopy to verify the expression of membrane and junctional markers (HEA, JAM-A, ZO-1 and Villin).

RESULTS: After 12 days of static culture, Caco-2 confluent monolayers were achieved. Then, the “open-well” static modules were placed in the “closed-well” perfusion module and cultured for 7 days in dynamic conditions and monitored every 2 days with bright field microscopy. At the end of the culture, the cartridges were easily retrieved and fluorescence microscopy allowed to compare junctional markers and cell density to the respect of static controls. The Arduino-based control unit allowed to control up to 18 perfusion modules independently and simultaneously.

DISCUSSION & CONCLUSIONS: TTOP modular concept was demonstrated, enabling simple preparation and advanced dynamic conditions for intestinal barrier tissue in-vitro replication. The optical access to both sides of the hosted sample enable to carry out live imaging studies (e.g. absorption of drugs, circulating immune cells) under dynamic controlled conditions. Moreover, the compact and the scalable design will allow to increase the number of devices controlled simultaneously, increasing the final throughput.

ACKNOWLEDGEMENTS:

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Biomechanics / biophysical stimuli and mechanotransduction

PP-117

Combining Computational Analysis with Organ-on-a-chip Intestine Model

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INTRODUCTION: Inflammatory bowel disease (IBD) is an umbrella term for chronic inflammatory conditions of the gastro-intestinal tract which include Crohn's disease and ulcerative colitis. Contributors to the development of IBD are thought to include environmental factors (stress, diet, smoking) and genetic susceptibility [1]. Due challenges in replicating the complex cellular interactions and microenvironment, IBD remains an idiopathic disorder that affects 6.2 million people globally [2]. Moreover, effect of fluid shear stress and mechanical cues generated by intestinal movement on cellular interactions remain elusive [3]. Here we generated a mathematical model of human epithelial barrier that provides spatial shear stress distribution. Our results suggest that the increasing shear stress damages the epithelial layer when not protected by intestinal mucus. We anticipate our results to be a starting point for developing in-depth understanding of the relationship between the mucus layer, shear stress and intestinal cell behaviour.

METHODS: We used X-ray, MRI and histology data to generate 3D intestinal crypt and mucus layers in COMSOL Multiphysics. Navier-Stokes equations were used in computations. PDMS based organ-on-a-chip systems were used to generate an in vitro model. Caco-2 cells were used as a biological component in the system. Immunofluorescence imaging and TEER measurements were used to assess epithelial barrier integrity.

RESULTS: Fluid shear stress on epithelial cells varied between 0.1 and 7.8 mPa depending on the position of the cells in the crypt which was reduced significantly with the mucus layer on the epithelial layer. Relative fluorescence and TEER values confirmed that the increasing shear stress had a negative effect on Caco-2 cells.

DISCUSSION & CONCLUSIONS: Intestinal epithelium experience constant fluid shear stress on their apical surface which can alter both function and behaviour. In order to better understand effect of mechanical stimuli, in-depth analysis of mechanical forces in the microenvironment is required. We demonstrate a proof-of-concept device supported by a computational model that is built with clinical data. We believe that this combined model can be used to recapitulate complex mechanobiology of the human intestine.

ACKNOWLEDGEMENTS: This work was supported by Science Foundation Ireland 18/EPSC-CDT/3583 and the Engineering and Physical Sciences Research Council EP/S02347X/1. CÚRAM is co-funded by the European Regional Development Fund and Science Foundation Ireland under Ireland's European Structural and Investment Fund Grant Number 13/RC/2073

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Keywords: In silico models, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-118

A novel ex-vivo model for studies of lung tissue repair

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INTRODUCTION: Current strategies used for treating lung diseases are merely symptom-relieving and do not actively repair injured tissue. Common degenerative lung diseases such as chronic obstructive pulmonary disease (COPD) arise from inadequate tissue repair processes. COPD alone accounts for 3 million deaths per year and is projected to be the third highest cause of deaths worldwide by 2030¹. A promising biological approach to repair lung damage is by manipulating the factors controlling lung generation during development². We have established a novel ex-vivo tool, the Acid Injury and Repair (AIR) model to facilitate studies of lung repair.

METHODS: A spatially restricted area of mouse precision-cut lung slices (PCLS) was injured using hydrochloric acid (HCl) providing a region of injured tissue adjacent to uninjured tissue. Tissue repair was assessed using fluorescent antibodies against relevant indicators of repair: proliferation (Ki67) and progenitor cells (pro-surfactant protein C, proSP-C and Transmembrane 4 L6 family member1, TM4SF1). The percentage of cells expressing repair indicators was quantified and compared in injured and uninjured regions of PCLS. Acid-injured PCLS were treated with potential activators or inhibitors of repair followed by quantification of the repair indicators (Ki67 and proSP-C).

RESULTS: The proportion of progenitor cells increases significantly (from 10.5 % to 16.6 %) in the injured region of tissue 48hr after acid administration. Interestingly, the percentage of Ki67 positive cells did not increase in the injured region but instead there was a significant increase of Ki67 in the uninjured region of lung tissue (from 8.6 % to 12.8 %) at 48 hr. Treatment with Wnt5a led to a further increase in proSP-C positive progenitor cells in the injured tissue area (16.1 % vs 20.5 %) whereas treatment with the Wnt inhibitor C59 almost completely inhibited the injury induced increase in progenitor cells back down to levels in uninjured control tissue (14% compared to 24% in control).

DISCUSSION & CONCLUSIONS: Ki67 and proSP-C provide robust readouts of repair in mouse PCLS at 48 hr post-injury. ProSP-C, a marker of endogenous progenitor cells, increased significantly upon injury. The increase in Ki67 in uninjured regions distant from the injured area of PCLS suggests cell proliferation in tissue surrounding the injury stimulated by paracrine factors may be a mechanism of tissue repair. We have established a novel ex-vivo model to study lung injury and repair, which can be employed to investigate cellular and molecular mechanisms of repair.

ACKNOWLEDGEMENTS:

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2.Stabler and Morrissey, *Cell Tissue Res* 2017,367(3):677.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Disease models

PP-119

Compartmentalized Chip for Improved Formation of Human Cardiac Spheroids Using Simulated Microgravity

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INTRODUCTION: Future human space exploration is threatened by negative health effects of prolonged exposure to the space environment. Cardiovascular effects have been identified as one of the main health risks, potentially jeopardizing future space missions. Human cardiac three-dimensional (3D) models play a crucial role in understanding the biological effects during long-term missions. Recently, evidence suggesting microgravity (μg) aids pluripotency maintenance and cardiac differentiation of human induced pluripotent stem cells (hiPSCs) has emerged. Furthermore, μg and simulated microgravity (s- μg) can aid the formation of 3D structures. However, the generation of these structures under s- μg is so far uncontrolled and inconsistent. Therefore we set out to design a device to control the formation of cardiac 3D models using s- μg .

METHODS: We prepared spheroids using different compositions of human cardiomyocytes, cardiac fibroblasts and endothelial cells at normal gravity (1g). Through immunofluorescent (IF) imaging and scanning electron microscopy (SEM), we determined the composition which most accurately represented the human heart (which was used for further experiments). Continuing, we designed and fabricated a compartmentalized chip through 3D-printing to control the formation of cardiac spheroids at s- μg using the random positioning machine (RPM). We thereafter compared spheroids formed at 1g and s- μg during 24h and 72h with the help of IF imaging, SEM and RNA sequencing.

RESULTS: From IF and SEM images, we observed an optimal composition of 70% cardiomyocytes, 20% cardiac fibroblasts and 10% endothelial cells, based on spheroid morphology, size and expression of cell type specific markers. We further designed and fabricated a 3D-printable chip which successfully aided the formation of cardiac spheroids with consistent size and shape, using s- μg . We observed morphological differences between the spheroids formed at 1g and s- μg , using both bright field and SEM images. Furthermore, molecular differences related to cell growth, mechanosensory pathways and extracellular matrix remodeling were seen at the global gene expression level.

DISCUSSION & CONCLUSIONS: Considering the already promising applications of μg in terms of 3D structures, generation of these in a controlled and scalable manner will be crucial for tissue engineering applications. In this study, we showed that our 3D-printable chip could be used for the formation of cardiac spheroids with consistent size and shape by means of s- μg using the RPM. Furthermore, we show molecular differences between 1g and s- μg spheroids central to tissue formation. All in all, this supports the use of our chip to enable spheroid formation at μg and its advantages here on Earth.

ACKNOWLEDGEMENTS:

REFERENCES:

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Cardiovascular

PP-120

Modeling the cellular diversity of the human heart in engineered heart tissue

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INTRODUCTION: Cardiomyocytes (CMs) are the most investigated cell type in the human heart.

However, there has been increasing evidence demonstrating the importance of non-myocytes in cardiac physiology and pathophysiology. Thus, the aim of this study was to generate multi-cell-type engineered heart tissues (EHTs) containing five cardiac cell populations i.e., CMs, endothelial cells (ECs), macrophages (Mφs), fibroblasts (FBs) and smooth muscle cells (SMCs).

METHODS: All differentiations were initiated by mesoderm induction followed by cell-type-specific stimulations. In short, CMs were generated by Wnt signaling inhibition, whereas ECs required VEGF treatment. SMCs and FBs were differentiated by activating the Wnt pathway followed by stimulation with TGFβ+bFGF or bFGF alone. Finally, Mφs were generated by treatment with IL-3 and M-CSF.

The expression of cell-type-specific markers was examined by flow cytometry and immunofluorescence. Characterized iPSC derivatives were cast into fibrin-based EHTs containing 60% CMs, 20% ECs, 10% Mφs, 5% FBs, and 5% SMCs. The optimal medium composition as well as construct properties were defined based on cell survival and remodeling of the EHTs. Additionally, the functionality of the tissues was assessed by automated contractility measurements.

RESULTS: The expression of cell-type-specific markers was demonstrated in all iPSC-derived populations - cTnT in CMs, CD31 in ECs, CD68 in Mφs, VIM in FBs, and MYH11 in SMCs. In order to determine the appropriate cell number per construct, two setups were established i.e., 1.67 million cells/EHT (1 million CMs) and 1 million cells/EHT (0.6 million CMs). In both analyzed setups, multi-cell-type EHTs developed and remodeled faster than the CM-only EHTs. However, increased cell number in the multi-cell-type EHTs caused elevated remodeling (58% shorter diameter than CM-only EHTs on 1st day of culture) and premature detachment from the silicone posts. As a result, the fibrin concentration in EHTs has been doubled to decelerate the remodeling process. While a higher fibrin content led to greater stability of the tissues, it also impaired their contractile properties and decreased the resting length by 12% of the multi-cell-type constructs with 0.6 million CMs. It was therefore determined that the lower cell number per EHT and standard fibrin concentration were the optimal parameters for the multi-cell-type tissues.

DISCUSSION & CONCLUSIONS: We have successfully established a stable and functional multi-cell-type system that allows the incorporation of five different cell types forming an in vitro cardiac system. The composition of multi-cell type EHTs more closely resembles native heart than uni-cell-type systems and will allow for multilineage cardiac disease modeling.

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REFERENCES:

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Cardiovascular

PP-121

Using an Ex Vivo Rat Aortic Ring Assay to Assess the Angiogenic Capacity of Adipose Derived Substrates

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INTRODUCTION: Soft tissue loss due to necrosis, malignancy or infection remains a significant cause of disability, with limited treatment options. An off-the-shelf, angiogenic soft tissue filler that can integrate with host vasculature would enhance implant biocompatibility and longevity. Adipose tissue can be processed or minimally processed to create potential soft tissue fillers. This project aims to produce both processed and minimally processed adipose tissue derivatives and test their angiogenic effect in an ex vivo rat aortic ring assay.

METHODS: Porcine decellularised adipose tissue (DAT) hydrogel was created and analysed for DNA, protein, and VEGF content. Lipoaspirate was harvested and Lipogems® technology was used to microfragment human adipose tissue. Each derivative was mixed with gelatin methacrylate (GelMa), an adipose-derived hydrogel, and incorporated into an ex vivo rat aortic assay. The number of endothelial sprouts, day of first sprout as well as the distance and rate of migration were quantified. The cellular composition of angiogenic sprouts was confirmed via immunohistochemistry.

RESULTS: DAT was shown to retain VEGF along with a significant concentration of extracellular proteins (6.03 mg/mL) and minimal DNA residue (138ng/mg). The Lipogems®/GelMa group produced vastly more angiogenic sprouts (221) than the Lipoaspirate/GelMa (51), DAT/GelMa (5), and GelMa (0) groups. It also induced earlier angiogenic sprouting than Lipoaspirate/GelMa ($p < 0.05$) and DAT/GelMa ($p < 0.1$). There was no statistically significant difference in distance or rate of cellular migration ($p = 0.9312$). Endothelial and mural cells were present, with a clear endothelial network established in the Lipogems®/GelMa group.

DISCUSSION & CONCLUSIONS: The DAT protocol used to produce DAT from porcine adipose tissue was able to retain VEGF, and important biological molecule in angiogenesis. It also exhibited good levels of extracellular matrix protein retention and an adequate reduction in DNA levels. GelMa was able to support angiogenic sprouting, as opposed to the collagen gels usually used in the literature. Lipogems enhanced angiogenesis when compared to lipoaspirate and a DAT hydrogel, without increasing cellular migration rate. Adipose-derived substrates have pro-angiogenic properties and may serve as effective soft tissue fillers by improving integration with host vasculature.

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REFERENCES:

Keywords: Vascular systems / vascularisation and heart, Hydrogels and injectable systems

PP-122

Highly scalable and automation-compatible organ-on-chip platform

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INTRODUCTION: Recent developments in organ-on-a-chip (OoC) models have provided unprecedented tools to control cell organization and interaction in 3D culture and emerged as a powerful predictor of disease progression. However, the obstacle in general implementation of OoCs is the absence of high throughput and instrumentation-free systems.

With these shortcomings in mind, we present a commercially available, standardized, high throughput OoC platform termed the AKITA® Plate. The platform allows a relevant modeling of vascularized organs and biological barriers (blood-brain, epithelial-endothelial), bone marrow niche, and organoid/spheroid culturing, resulting in an easy-to-use system compatible with standard multiwell plate formats. It enhances robustness and reproducibility while maintaining high throughput and important biological utilities like shear flow and biomimetic surface stiffness.

METHODS: The AKITA® Plate is designed for plug & play workflows, thus ensuring ease of use, robust data collection, and saving of time and resources. AKITA® Plate has a standard 96/384 well plate format with 24 or 96 individual culture units, which enables automated handling and confocal imaging. With an integrated microporous membrane to separate the apical and basolateral culture chambers, this platform allows establishment of a co-culture with static conditions in one compartment and dynamic conditions in the other. The AKITA plate allows the test of compound permeability, and the monitoring of barrier tightness either with fluorescent probes, confocal imaging or by measuring electrical resistance with AKITA® Lid device.

RESULTS: The AKITA® Plate has the ability to improve the structural and functional complexity of currently existing human organ models, such as barrier, air-liquid interface, and vascularized organoid models. With AKITA® Plate, we have managed to demonstrate in vivo-like permeabilities for the blood-brain-barrier as well as the gut barrier. Moreover, shear stress by dynamic flow has been demonstrated to increase barrier tightness and the polarization of the models.

DISCUSSION & CONCLUSIONS: In conclusion, we established a commercially available high throughput microfluidic platform for cell-based in vitro assays that are flexible to various organ models and diseases. With our standardized and translational platform, we provide a better alternative to animal testing, and most importantly, accelerate therapeutic studies as well as drug discovery processes.

Keywords: Scaling up & manufacturing,

PP-123

Advancing the knowledge in Polycystic Kidney Disease (PKD) using organoids as a personalized medicine platform

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INTRODUCTION: Kidney organoids and tubuloids have been suggested as promising alternative models for patient-oriented therapeutic screening [1]. ASC-derived human kidney tubuloids recapitulate the renewal of adult kidney epithelium, embodying the proximal tubule, loop of Henle, distal tubules, and collecting duct, as confirmed by the expression of different proteins [2]. They mirror the in vivo nephron more closely than traditional cell lines and allow studies of human tissue in a high throughput fashion, in contrast to animal models [3]. A major restriction for performing patient-derived organoid cultures, including tubuloids, is the availability of fresh tissue. However, several reports have shown that urine can serve as a source for kidney epithelial cells [4], truly recapitulating the donor genotype and phenotype [2].

METHODS: We are establishing tubuloids cultures from healthy donors at our lab, following the protocol by Schutgens and colleagues [2]. Briefly, urine samples are collected, centrifuges and washed with culture medium 3 times. Afterwards, the pellet is resuspended into Matrigel and domes are formed in 24 well plates. After 1 -2 weeks organoids formation is visible. These structures are currently being characterized by histology and confocal microscopy. Once the characterization is complete, we will move for the establishment of tubuloids from ADPKD patients, envisioning to obtain a simple in vitro model of the disease that allow us to study its underlying cellular and molecular pathophysiology.

RESULTS: Confocal microscopy of the human tubuloids revealed the presence of cells from the tubular epithelium, and allowed to distinguish the formation of tubular structures within the organoid. More characterization is being performed currently, namely by RT-PCR and histology.

DISCUSSION & CONCLUSIONS: Once the characterization is complete, we will move for the establishment of tubuloids from ADPKD patients, envisioning to obtain a simple in vitro model of the disease that allow us to study its underlying cellular and molecular pathophysiology.

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Keywords: Personalised medicine, Multipotent (mesenchymal) stem cells

PP-124

Development of a novel human Type 1 Diabetic 3D disease model for Diabetic wounds, mimicking in vivo niche

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INTRODUCTION: Diabetic wounds (DW) are a common complication of diabetes (DM) that can lead to limb amputations. Impaired wound healing, characterized by a delay in cell migration, decreased angiogenesis and prolonged inflammation, and secondary infections make its current treatment challenging and expensive. DW disease models are valuable tools to study the pathophysiology of the diabetic wound environment and to evaluate candidate therapeutic agents. However, 2D in vitro models do not adequately replicate in vivo pathophysiology. For this reason, models that can replicate the complex pathophysiology of DW need to recapitulate the in vivo 3D environment and comply with the 3R's (refinement, reduction, and replacement) in research. To fulfil these, our model involves the use of the CAM assay, within the first two thirds of gestation, to spare the use of more sentient animals.

METHODS: Human dermal fibroblasts (HDF) and keratinocytes (HaCaT) cultured in 2D and 3D were exposed to hyperglycaemic media to reproduce cell behaviour and morphological changes in diabetes. Angiogenic response of 3D skin mimicking scaffolds seeded with HDF and HaCaT was evaluated using the chicken embryo chorioallantoic membrane (CAM) assay of healthy and Type 1 Diabetic chicken embryos (T1DCE). T1DCE were created by selectively knocking out pancreatic β cells using streptozotocin (STZ). This outcome was confirmed by measuring blood glucose levels and by immunofluorescent staining against pancreatic markers PDX-1 and Neurogenin 3.

RESULTS: Exposure to hyperglycaemic conditions delayed migration of primary derived fibroblast cultured in 2D. Moreover, higher glucose concentrations increased the nuclear size of both cocultured cell types (HDF and HaCaT) in the 3D model.

The creation of diabetic chicken embryos by using STZ was confirmed with an absence in fluorescence of the pancreatic markers PDX-1 and Neurogenin 3, and an increase in blood glucose levels in the STZ treated embryos (7.6 ± 0.33 mmol/L), when compared to the control (5.3 ± 0.49 mmol/L).

A decrease in angiogenesis was observed in normoglycemic 3D scaffolds placed on CAM of STZ T1DCE suggesting that this effect is related to the systemic effect of high glucose rather to the one of its microenvironment.

DISCUSSION & CONCLUSIONS: The model using 3D scaffolds placed onto CAM of diabetic chicken embryos was able to replicate key aspects of a diabetic pathophysiology (delayed migration and decreased angiogenesis). Our developed 3D model can serve as a powerful tailored tool for future therapeutic drug testing as it mimics in vivo environment of DW and fulfils the 3R's in research.

Keywords: In vitro microenvironments, Diabetic healing

PP-125

Generation of Multicellular, Immunocompetent, 3D Liver Organoids Using Human Induced Pluripotent Stem Cells to Model NAFLD

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INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is one of the most crucial liver disorders through the world, especially in western countries, and there is no clinical drug approved for its treatment yet. In addition, the pathophysiological mechanisms that cause NAFLD have not been defined clearly. Therefore, the need for in vitro disease models that more accurately reflect NAFLD remains. In this work, we aimed to generate three dimensional (3D) human liver spheroids consisting of parenchymal and nonparenchymal liver cells, and immune cells to establish steatosis and fibrotic conditions.

METHODS: Human induced pluripotent stem cells (hiPSCs) derived from patients were differentiated into hepatocyte-like cells (HLCs) and cholangiocyte-like cells (CLCs) organoids using differentiation protocols developed and optimized by Hannan Group. hiPSCs-derived HLCs were treated with different concentrations of palmitic acid (PA) for 48 hours to induce steatosis. HLCs and CLCs were characterized by assessing the expression levels of mature liver and cholangiocyte-related genes via quantitative real time polymerase chain reaction (qPCR). After PA treatment, lipid accumulation was monitored by high content imaging and staining.

RESULTS: During HLC differentiation, the morphologies of hiPSCs started to change remarkably from the 1st day of hepatic maturation (HM) stimulation and on the last day of maturation (day 10), they had a hexagonal structure like primary hepatocyte cells. The expression of mature liver-related genes gradually rose during HM and reached the maximum level on the 10th day of maturation. In terms of CLCs differentiation, CLCs organoids started to grow on the 3rd day and their size gradually expanded for 10 days. Additionally, they had cystic or branching morphologies. After 10 days of maturation, the organoids continued to express their biliary genes. The expression of AFB (hepatic marker) was significantly reduced throughout the differentiation. Our results showed that 300 μ M PA was sufficient to induce lipid accumulation and steatosis in HLCs.

DISCUSSION & CONCLUSIONS: We were achieved to generate HLCs and CLCs organoids derived from patient hiPSCs. Additionally, steatosis phase was accomplished using 300 μ M PA treatment. The use of hiPSCs derived from patients to model NAFLD in vitro may provide a better understanding of the pathophysiological molecular mechanisms causing the development and progression of the disease. Thus, it may allow the discovery of more effective personalized treatment methods.

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Keywords: Induced pluripotent stem cells, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-126

Creating a 3D Human Engineered Heart Tissue Fibrosis Model – Technical Perspectives

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INTRODUCTION: Heart disease often involves pathological fibrosis, which requires fibroblast activation. Activated enhancer sequences could be disease specific treatment targets. Here, we created a fibrosis model in engineered heart tissue from human induced pluripotent stem cell-derived cardiomyocytes (C) and fibroblasts (F; CF-EHT) to study the consequences of enhancer manipulation.

METHODS: In 24-well fibrin based CF-EHTs composed of 1 Mio cells each (9:1 C and quiescent F), cultured for 22 days, we evaluated TGF- β 1 (10, 20 and 48 ng/ml) for fibroblast activation and the TGF- β 1 receptor inhibitor, SB-43152 (1, 5 and 10 μ M) for maintaining quiescence. Different time points of treatment onset (d0, d3, d7 and d13 of culture) and of serum withdrawal (d3 or d14) were evaluated. Readouts included morphology, contractility, gene expression and confocal microscopy imaging.

RESULTS: Force was not decreased by TGF- β 1 or SB-43152 treatment in any experiment. Structural remodelling was slower in CF-EHTs with early SB-43152 treatment at each concentration, but was not affected by TGF- β 1 treatment, regardless of concentration and treatment onset. Serum reduction at d3 from 10% to 0.5% led to higher final force than in CF-EHTs with 10% serum (e.g. 0.12 vs 0.07 mN, >4 CF-EHTs per group at d19). In an experiment with treatment onset on d13, fibrosis-related markers *COL1A1*, *CCN2* and *ACTA2* mRNA were ~0.5x in CF-EHT inhibited with 5 μ M SB-43152, $\geq$ 5x in CF-EHT activated with 10 ng/mL TGF- β 1, and $\geq$ 3x in CF-EHTs cultured with 10% serum, compared to respective mRNA abundance in untreated CF-EHT in 0.5% serum. Preliminary results showed no obvious differences in contractility and gene expression in CF-EHT with enhancer-deleted fibroblasts.

DISCUSSION & CONCLUSIONS: Our EHT-based fibrosis model has been engineered for optimal contrast between quiescent and fibrotic conditions. This was best achieved by i) reducing serum from 10% to 0.5% from d3 on, and ii) additional treatment with 5 μ M SB-43152 from d10 to d13 in all desired CF-EHT groups, to achieve quiescent baseline conditions. Subsequent treatment could then be carried out at d13 (10 ng/mL TGF- β 1 or high serum concentration (both activated), continuous SB-43152 treatment (quiescent) or no treatment (control)). We used our model to evaluate consequences of pro-fibrotic enhancer deletion, so far without overt differences. In the future, activation pathways other than the TGF- β 1/SMAD pathway will be addressed by stretch and/or adrenergic stress.

Keywords: Cardiovascular, Induced pluripotent stem cells

PP-127

Composite 3D human cardiac minitissues are a representative model of anthracycline cardiotoxicity

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INTRODUCTION: In spite of the incremental success of modern anti-cancer therapies, cardiotoxicity has been a rising concern, striking even years after the termination of the therapy. This is especially relevant for children, where an increased risk of up to 10 times has been reported. Crucially, research for mechanisms and potential prophylactic options is hampered by the lack of representative in vitro models. Human induced pluripotent stem cells (hiPSC) can give rise to cardiomyocytes (CMs) with immature features, making them highly suitable for this purpose. However, the lack of a 3D multicellular environment diminishes their relevance. In this work, we combined hiPSC-derived cardiac phenotypes with 3D printed scaffolds to generate 2 models of anthracycline toxicity, with single or repeated exposure to doxorubicin (doxo).

METHODS: Cardiac engineered tissues were built using hiPSC-CMs and cardiac fibroblasts (hiPSC-CFs), in an 80:20 proportion. These cells were embedded in a fibrin hydrogel and casted on melt-electrowritten (MEW) PCL scaffolds. To mimic different exposure regimens, minitissues were exposed to 0, 250 or 2500 nM doxo 7 days after generation. These concentrations recapitulate the range seen in treated patients. In the single exposure group, the drug was added once, whereas in the repeated exposure, it was provided for 7 consecutive days. Functionality (beating rate), structure, cellular proportions and gene expression were analysed for up to 1 week.

RESULTS: Tissues built with MEW and hiPSC-derived cardiac cells showed a stable functionality, with beating rate stabilizing around 0.5-1 Hz. Human iPSC-cells displayed a normal phenotype, with prominent striations (CMs). The repeated exposure to doxo (7 days) abrogated functionality as beating ceased. Importantly, single exposure to 2500 nM doxo similarly caused cessation of bating, whereas 250 nM significantly decreased beating rate. The structure of CMs was also altered, with most sarcomeres absent or disarranged on the 7-day model (more important for the higher concentration). This effect was also present in the single dose model for the 2500 nM group, but was less prominent (albeit significant) for tissues treated once with 250 nM doxo. At the gene expression level, repeated addition of doxo for 7 days caused a marked reduction of all genes screened, likely caused by the huge cell death present.

DISCUSSION & CONCLUSIONS: MEW-based human bioengineered myocardium is a promising model for anthracycline-based cardiac toxicity, to be extrapolated to drugs such as tyrosine kinase inhibitors, or radiation. The application of next generation sequencing technologies is expected to shed mechanistic light on this pathological event.

Keywords: Cardiovascular, Disease models

PP-128

Development of silk fibroin-based tri-culture 3D in vitro model of breast cancer for high throughput anticancer drug screening

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INTRODUCTION: Breast cancer (BC) is characterized by the presence of cancerous lump and different type of stromal cells surrounding cancerous mass. Cross-talk between cancer and stromal cells provide important signalling cues that help in further proliferation of cancer and promote metastasis. Hence, to build the BC model, considering cellular heterogeneity and distribution of multiple cell type is crucial. Traditional 2-dimensional (2D) models fail to recapitulate such environment hence drug testing results are not accurate and considered to be major pitfall during pre-clinical evaluation of anticancer drugs. In this regard, developing a 3-dimensional (3D) in vitro BC model providing adequate cellular interaction and heterogeneity is preferred to understand accurate drug response in realistic cancer microenvironment.

METHODS: The stromal heterogeneity of BC is mimicked by using adipocytes and endothelial cells seeded on porous 3D SF scaffold. Whereas BC mass is created by encapsulating MDA-MB-231 cancer cells in gelatin methacryloyl (GelMA) hydrogel respectively. Both, cancer and stromal compartment is combined together to facilitate cancer-stromal cell cross-talk. The robustness of the developed tri-culture (TC) model is validated extensively by cellular proliferation study, gene expression and drug screening.

RESULTS: The developed model demonstrated good proliferation and viability of cancer and stromal cells for 14 days in the respective matrices. Endothelial cells were forming tube-like structure which was confirmed through phalloidin staining. Histological staining showed positive staining for oil red o and vWF for adipocytes and endothelial cells respectively. Gene expression study of the TC model demonstrated elevated expression of N-cadherin, VEGF, IL-6 and MMP-9 and decreased expression of epithelial marker E-cadherin from day 1 to day 7 of culture. MTT assay on the TC model demonstrated higher IC₅₀ for doxorubicin and cis-platin as compared to mono-culture of cancer cells.

DISCUSSION & CONCLUSIONS: The result obtained from histological staining and gene expression study validated the tumor enhancing role of stromal cells. This shows the cross-talk and signalling cues between cancer and stromal cells were preserved in the developed model. The current model mimicking realistic cancer microenvironment have potential advantage to be used for pre-clinical anticancer drug screening.

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Keywords: In vitro microenvironments, Cancer

PP-129

Perfusion bioreactor based 3D printed in vitro liver model for drug screening applications

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INTRODUCTION: Drug-induced liver injury (DILI) is a multifactorial pathophysiological process that causes acute liver failure. The poor predictability of DILI has led to high attrition rates of drug candidates at the clinical and post marketing authorization stages. Inability to predict DILI is largely due to both discordance between human and animal adverse drug reactions and lack of pre-clinical models. The limited predictivity of DILI in preclinical trials can be remediated using 3D engineered liver models. For improved drug screening platforms in the preclinical trial phase, in this study we developed and validated a microscale bioreactor-based 3D printed liver model conducive to hepatotoxicity testing.

METHODS: A hybrid microscale perfusion device was designed and fabricated using acrylonitrile butadiene styrene and polydimethylsiloxane. Computational Flow Dynamics (CFD) simulation was performed to determine the flow characteristics which mimicked the in vivo blood flow conditions of the human liver sinusoid. Further, 3D printing of biomimetic liver scaffolds was performed using a developed silk-based biomaterial ink. Rheological and physicochemical characterization of the developed biomaterial ink and acellular scaffolds were performed. The physiological similarity of the parenchymal and non-parenchymal cells based liver model was verified in the dynamic condition in terms of cell viability, arrangement and functions. Furthermore, the liver model was also evaluated for drug toxicity assessment following 7 days exposure to different concentrations of non-hepatotoxicants, idiosyncratic hepatotoxicant and clinical hepatotoxicant.

RESULTS: The low velocities obtained from CFD analysis suggested that the mass transfer of media in the liver model is expected to be dominated by diffusion and the flow characteristics mimicked the in vivo blood flow pattern of the hepatic sinusoids. Further, the developed silk-based biomaterial ink portrayed suitable printability and rheological attributes. The 3D printed scaffolds supported the viability and proliferation of both parenchymal and non-parenchymal cells. The physiometric liver model resembled the native cords of hepatocytes with a functional hepatic portal-like vein and sinusoidal lumen-like network with enhanced albumin production, urea synthesis, and cytochrome P450 activity. Furthermore, the drug toxicity assessment revealed a dose-dependent clinically

relevant hepatotoxic response.

DISCUSSION & CONCLUSIONS: The physiomimetic bioreactor-based liver model presented here closely recapitulated the in vivo liver microarchitecture, function, and drug reactions of human tissues. The study highlighted promising results for long-term culture of both parenchymal and non-parenchymal cells as well as drug toxicity testing.

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REFERENCES: Janani, G., et al. (2022). ACS Applied Materials & Interfaces, 14(8), 10167-10186

Keywords: 3D printing and bioprinting, Bioreactors

PP-130

Engineering Biomimetic Three-Dimensional Microvascular Microenvironments For Use Within Organ-On-A-Chip Platforms

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INTRODUCTION: The three-dimensional (3D) microenvironment in which cells reside influences their behaviour and maturation via mechanobiological cues. Cues such as topography and stiffness are important when modelling a vasculature within organ-on-a-chip models to allow for a greater accuracy of the modelling of diseases. Polymeric microparticles can be designed to deliver specific 3D topographical cues.

METHODS: Poly(lactic-co-glycolic acid) microparticles were topographically modified using the phase separation of a sacrificial component to create dimpled (golf ball-like) microparticles (DMPs), which were compared to conventional smooth microparticles (SMPs). Human Endothelial Umbilical Vein Cells (HUVECs) were seeded onto microparticles and assessed for their viability (Live/Dead staining), proliferation (Ki67-staining), and presence of endothelial cell-cell junctions (CD31 staining).

Fluorescence images of Ki-67 and CD31 immunostaining were analysed with CellProfiler, an automated image analysis platform. Optimal microparticle designs were applied within microfluidic devices. Flow simulations were conducted on COMSOL to determine the velocity within microfluidic devices to study the behaviour of the cell-microparticle aggregates within a dynamic system.

RESULTS: HUVECs show a high level of viability on both SMPs and DMPs over 5 days, displaying HUVEC organisation along the SMPs indicating early microvascular network formation on SMPs only. Despite higher proliferation of HUVECs on the DMPs at day 2 relative to SMPs, the proliferation of 3D-cultured HUVECs decreased on the DMPs five days post seeding compared to SMPs. Additionally, HUVECs cultured on SMPs displayed an increase in endothelial cell junctions when co-cultured with mesenchymal stem cells (MSCs). DMPs-cultured HUVECs did not change in their expression of endothelial cell junctions when co-cultured with MSCs. Velocity simulations showed that a microfluidic device with a channel design that has a volume of 0.58mm³ and a surface area of 0.839mm² will support laminar flow through the device. This can be filled with microparticles providing a surface area for cellular attachment of 1.83cm².

DISCUSSION & CONCLUSIONS: HUVECs can be successfully cultured on microparticles to provide a 3D microenvironment that mimics the in vivo basement membrane structure. Current work is being carried out to incorporate these 3D cultures into microfluidic devices to allow for the studying of shear stress on the HUVECs-microparticle aggregates and study any synergistic effects between the biomechanical cues provided by the microparticles and fluid flow. This model can provide a physiologically relevant culture for studying a range of disease processes, e.g., metastasis through the vasculature.

Keywords: Biomaterials, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-131

Modulation of macrophage polarization induced "in vitro" by 3D micro scaffolds

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INTRODUCTION: The implantation "in vivo" of a polymeric micro scaffold triggers the immune system, by orienting macrophages towards the M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes. These events evolve into a foreign body reaction (FBR) [1] responsible for the fibrotic encapsulation or integration of the implant, respectively. In this work, we optimized the design of a 3D micro scaffold in its ability to guide macrophage polarization, with the aim to maximize its potential for integration after implantation "in vivo".

METHODS: We performed fabrication of the 3D micro scaffolds by 2-photon polymerization of the SZ2080 biocompatible photosensitive resin, as described before [2]. We designed scaffold architectures consisting of microgrids with increasing pore dimensions, ranging from 10x10x15 μm^3 to 50x50x20 μm^3 . We cultured human macrophages with different phenotypes (M0, M1, and M2) in the microstructures [3]. After 48 hours, we investigated the level of macrophage polarization by immunofluorescence staining and Western blot. We incubated macrophages with 1 μM of SiR-actin fluorogenic probes and we used time-lapse confocal imaging to assess how the macrophages cytoskeleton functionally adapts in presence of the microstructure.

RESULTS: Cell culture experiments allowed to appreciate how different 3D micro geometries modulate macrophages activity. Pro-inflammatory/anti-inflammatory protein expression evaluation demonstrated that pore geometry significantly influences macrophages polarization. Among the several micro scaffold geometries tested, we identified the microstructure better reducing the pro-inflammatory macrophages response.

DISCUSSION & CONCLUSIONS: Our results show that polarization towards M1 or M2 phenotypes is modulated by the different microstructure geometries tested, suggesting a need for integrating specific 3D geometrical features in the design of miniaturized implantable scaffolds, to potentially prevent their fibrotic encapsulation. Further investigation on how the macrophages cytoskeleton adapts and modifies in presence of the 3D microstructures will establish the role of the cytoskeleton in macrophage polarization. In future work, we will carry out additional assays to analyze specific molecules released in the cell culture supernatants to better corroborate our observations.

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Keywords: Immunity / immunomodulation / macrophage,

PP-132

Effect of Biomimetic Substrates and Extracellular Matrix Proteins on the Culturing, Activation and Differentiation of Skeletal Muscle Satellite Cells

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INTRODUCTION: Skeletal muscle satellite cells (SCs) are muscular progenitor cells and have therapeutic potential for myopathies by contributing to tissue regeneration. This process involves activation through bio-signalling pathways, migration, proliferation, and differentiation into mature skeletal muscle tissue cells. Understanding SC activation and differentiation can aid in developing treatments for skeletal muscle diseases such as muscle atrophy.

This study explored the influence of Poly-L-Lysine (PLL) and PhenoDrive-Y, substrates mimicking the stem cell niches' basement membrane and extracellular matrix proteins like Fibronectin, Laminin, and Collagen Type-1 (CT-1), on SCs' morphological features and marker expression of pluripotent and differentiated phenotypes. The study examined the effect of these substrates in the presence and absence of serum in the growth medium.

METHODS: Human SCs cultured in T75-flasks were coated with PLL. Four 24 well-plates were coated with 10mg/ml PLL, 0.1mg/ml PhenoDrive-Y, 1%(w/v) Fibronectin, 0.01%(w/v) Laminin and 0.01%(w/v) CT-1. Cells were seeded at a density of 1×10^5 cells/ml for 1, 4, 7 and 14 days in a specialised medium in absence and presence of 10% foetal bovine serum. SCs' growth and behaviour observed and analysed by visible light microscopy and related time-lapse microscopy before fixing. Later, biomarkers expression of: Pax7 (pluripotent cells), MyoD1 and Myf5 (cells at different stages of differentiation), Notch1 (proliferating cells) and Integrin- $\alpha 7$ (bioligand specific cell receptor) were analysed by immunostaining and observed by confocal fluorescence microscopy.

RESULTS: SCs have attached and adhered to all the treated well-plates. Starting from day 4, increased number of cells with a spreading morphology was observed on PLL. A negligible cell proliferation, but a more migratory phenotype and a morphology (fused sarcomere-like cells) and (MyoD1 and Myf5) expressions typical of differentiated muscle cells were observed on PhenoDrive-Y. Similar SCs' behaviour in their structure and growth were seen on Laminin and PhenoDrive-Y, while Fibronectin and CT1 behaved more like PLL.

DISCUSSION & CONCLUSIONS: The results showed that ECM proteins, such as laminin found in the basement membrane of the stem cell niche, endothelia, and epithelia, can stimulate SCs to migrate and differentiate into skeletal muscle cells. Similarly, cells cultured on PhenoDrive-Y, a substrate mimicking the structure of Collagen Type-IV and presenting laminin bioligands, had a similar effect. In contrast, PLL lacked specific ECM bioligands and ubiquitous connective tissue proteins, which only stimulated SC proliferation without significantly affecting differentiation. This study could inform the development of new biomaterials for tissue engineering scaffolds that can encourage SC proliferation or improve muscle repair efficiency by stimulating differentiation.

Keywords: Differentiation, Stem cell niche

PP-133

Co-assembling 3D *in vitro* models to improve personalized cancer treatments

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INTRODUCTION: Current three-dimensional (3D) *in vitro* models fail to recapitulate the complexity of the tumour microenvironment including recreating the extracellular matrix (ECM) and tumour-stroma interactions present in tumour progression and metastasis. Our goal is to develop molecularly complex, yet defined, 3D models that can be easily assembled to reproduce key aspects of the tumour architecture and composition. Our hope is that these models will enable a better understanding of the colorectal cancer progression and prediction of drug responses. For this purpose, we have developed a co-assembling platform using peptide amphiphiles (PAs) as organizers of ECM proteins to engineer composite (PA-ECM) nanofibrous hydrogels that can be tuned to recreate different tumour microenvironments (1,2).

METHODS: PAs were co-assembled with ECM proteins to produce hydrogels with specific molecular concentrations and defined mechanical properties. Two types of hydrogels were synthesized to investigate the effect of hydrogel nano and microstructure including co-assembled hydrogels comprising either randomly oriented (rPA-ECM) or aligned nanofibers (aPA-ECM) following an established annealing process prior to co-assembly (3). Human colorectal cancer cells (Caco-2) and organoids were embedded in these hydrogels and rheological and morphological measurements were characterized. Differences in cell survival, morphology, and phenotypic marker expression were analysed via a life/dead assay, confocal microscopy, and RT-PCR, respectively.

RESULTS: Fibrillar and compositional networks of the aPA-ECM and rPA-ECM hydrogels were characterized. Caco-2 cells growing in PA-ECM hydrogels were more viable, proliferative, and displayed higher levels of genetic differentiation markers compared to cells growing in aPA-ECM as evidenced by gene expression analysis and immunofluorescence microscopy.

DISCUSSION & CONCLUSIONS: We report a biomimetic 3D *in vitro* model that aims to resemble structural and compositional features of the colorectal tumour microenvironment. This approach aims to better understand colorectal cancer progression and better predict the response to treatment. Further integration of patient derived organoids with PA-ECM hydrogel will contribute to the complexity of tumour microenvironment for long-term culture of organoids. We aim to optimize this platform to more accurately model personalized cancer treatments.

ACKNOWLEDGEMENTS: This work was supported by the ERC PoC Novachip and EPSRC Case Studentship.

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Keywords: Biomaterials, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-134

Selectively-Coated Microfibre as High-Aspect-Ratio Flexible Organic Electrochemical Transistors for Biointerface Applications

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INTRODUCTION: Organic Electrochemical Transistors (OECTs) is a category of bioelectronic sensor with an intrinsic functionality of sensing electrophysiological signals in wet environments. They can be functionalized to sense other biological signals such as metabolites and membrane behaviours. Most state-of-art OECTs are fabricated on a planar substrate. However, since biological systems are often non-planar, the transitional steps of adapting planar devices to various applications become a source of significant engineering challenges. [1]

An alternative OECT design is fibres (fibre-OECT). These devices are usually formed by extrusion or coating on a separately manufactured core, providing a tailored integration into corresponding bio-related applications. However, the extrusion processes often contain chemically coupled materials, making it difficult to change; both coating and extrusion require a hazardous bath. [2-3]

METHODS: In this study, a type of fibre-OECT is manufactured with a novel selective coating technique. The core material is 23 μ m ϕ PET (Polyethylene terephthalate) fibres (Goodfellow). The coating formulation is based on PEDOT:PSS (poly(3,4-ethylenedioxythiophene) polystyrene sulfonate) (Clevios PH1000).

During coating, a fibre is affixed to a robotic gantry (Igus) and rotated axially. A coating material droplet (<10 μ L) is held with a syringe tip and dragged across the fibre repeatedly to create coating of an arbitrary length.

The manufactured fibre-OECTs are tested using semiconductor analyser Keithley 2612B.

RESULTS: The manufactured fibre-OECTs are depletion mode, have a characteristic response time of under 1ms and an on-OFF ratio of 66 (measured over a 2mm PBS droplet on a 2cm device).

The fibre-OECTs can survive soaking in phosphate-buffered saline for 4-8 weeks depending on formulation; best formulation device can be used to create curves and knots without functional failure or immediate degradation. Handling does not damage the coating in all experiments, but frictional force creates cumulative damage.

DISCUSSION & CONCLUSIONS: For a signal that affects a longer section of the full fibre-OECT channel, it will experience a higher sensitivity but a lower response time, and vice versa, which is consistent with Bernard Model [1]. They can be used to measure signals up to 1kHz and will be most suitable for applications requiring flexible and micro-bioelectronic sensors, such as volumetric sensing within a biological tissue.

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Keywords: Composite materials, Interfaces - engineered

PP-135

Developing a Porcine Ex-Vivo Living Limb System to Investigate the Bidirectional Control of Advanced Prostheses

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INTRODUCTION: Limb loss carries a personal and societal impact, and amputations are forecast to double by 2050 (Europe and US) from vascular disease and diabetes alone. Currently, the mainstay of management relies on an artificial prosthesis that does not look, move, or feel like a human limb. Ordinarily, the peripheral nervous system is responsible for transmitting bidirectional 'signals' either to target muscles (for volitional control) or from the environment to the brain (sensory feedback). A barrier to incorporating advanced biorobotic prostheses into clinical practice is understanding how these 'two-way' signals operate biologically.

METHODS: Animal model

We utilised an advanced, ex-vivo living limb system to restore tissue metabolism using a bespoke oxygenated, blood-based media, supported by critical substrates for health. The model is ethically advantageous given nerve stimulation triggers an unwarranted sensory perception and risks nerve injury. This is an ideal 3Rs approach, avoiding the need for a live animal model. Bilateral paired randomised forelimbs were subjected to either electrical stimulation (perfused for 24hrs via the axillary/radial artery at normothermia), or cold-storage (CSS). Arterial flow, pressure, and arterial blood gas analysis were recorded throughout (mean±SD).

Neuromuscular assessment:

The median nerve at the severed brachial plexus was stimulated to assess motor function. EMG signals were recorded from the median innervated Flexor Digitorum Superficialis (FDS) and Pronator Teres (PT). Compound muscle action potentials (CMAPs) were used to quantify muscle contraction using surface electrodes (Shimmer sensing™). Data was recorded using Consensys™ and analysed using Python™.

RESULTS: (n=8 bilateral limbs; 4 NMP vs 4 CSS)

Flow rate was stable throughout (303.3±69ml/min) and tissue perfusion was maintained in all NMP limbs using visual/thermal criteria. Lactic acidosis was physiologically buffered by the addition of bicarbonate (pH 7.45±0.1, base excess 0.54±3mmol/L) and calcium 0.95±0.1mmol/L and electrolyte concentrations remained stable. Glucose consumption was recorded throughout, with continuous physiological metabolic activity. Neuromuscular contraction was only observed macroscopically in the NMP limb after 50mins. Contraction for NMP was detectable until 9hrs whereas no activity was observed with CSS.

DISCUSSION & CONCLUSIONS: The early development of this ex-vivo model demonstrates that the porcine living limb perfusion system can be used as a model for efferent (motor) and afferent (sensory) testing of a bioprosthetic interface. We hypothesise that this model will allow the ethical, high through-put testing and have recently

Keywords: Composite materials, Interfaces - engineered

PP-136

From Preclinical to Clinical Translation of Intervertebral Disc Cell-based Regeneration - Effects of Species-Specific Scale, Metabolism and Matrix Synthesis Rates

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INTRODUCTION: After demonstrating promise preclinically, many cell-based therapies do not show similar efficacy under clinical evaluation. It is believed that the harsh nutrient microenvironment and “hostile” nature of the degenerating disc is linked to weak outcomes of prospective studies.[1–3] This work investigates the species-specific metabolic activity and regenerative capacity of a small and large animal model in an attempt to ascertain their scientific merit for successful clinical translation. **METHODS:** Metabolically active cell density was determined using MTT and DAPI[4]. Disc cells were isolated from rats, goats and patients undergoing discectomy. Tissue was obtained under informed consent and ethical approval. Cell spheroids were formed in an agarose microwell array, fabricated using a positive stamp. Spheroids were cultured under low glucose and 10% oxygen, informed through in-silico models. A Seahorse analyzer was used to monitor oxygen, a measure of oxygen consumption rate (OCR) and pH, a measure of lactate production rate (LPR). Spheroids were digested and rates were normalised by DNA. sGAG production was quantified after 14 days. 3D in-silico models were created through geometrical analysis and coupled reaction-diffusion kinetics.[4] Additionally, we developed pre-clinical GAG regeneration models, as employed previously for human.[5-6]

RESULTS: No significant differences in geometry was found between C5-6 and C8-9 in rat and only L5-6 was significantly different in goat (N=3). Rat have a 60-fold higher cell density than human, while goat is 4-fold higher (N=6). While no significance was determined between rat NP and AF, goat had a significantly higher AF density. All species formed stable spheroids within 5-7 days and retained high viability. Human cells have significantly lower OCR compared to animal cells, while only rat NP had significantly higher LPR than goat and human LPR (N=3). No significant differences were found for GAG synthesis rates among species (N=3).

DISCUSSION & CONCLUSIONS: These models address the scale-effect between different species by creating 3D geometries with experimentally determined active cell densities. Despite similar GAG deposition per cell, when these experimental parameters are modelled in-silico, the species-specific scale and cell density are the predominant factors to determining the species-specific microenvironment and regeneration capacity. This work shows that while 8 weeks is sufficient to achieve GAG deposition in rats, and 24 weeks in goat, > 10 years is needed for equivalent matrix

recovery in human. Therefore, it's unlikely that cell therapies alone are a viable treatment to curb degeneration and initiate regeneration clinically.

REFERENCES: [1] (Sakai+2015) [2] (Smith+ 2018) [3] (Farhang+2020) [4] (McDonnell+2021) [5] (Zhu+2016) [6] (Gu+2014)

Keywords: In silico models, Cell therapy

PP-137

Comparison of different nanofiber scaffolds effects on bone regeneration of calvaria defect in a Wistar rat model _Importance of porosity

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INTRODUCTION: Skeletal tissue injury is a major burden on the global healthcare system due to an aging population. In addition, metabolic disorders such as diabetes and osteoporosis further impede the healing process. Tissue engineering scaffold for reconstructive strategies offer exciting opportunities to overcome poor self-healing capacity of the skeletal tissue.

METHODS: We studied bone regeneration potential of cellulose-based nanostructured-biomaterials on a pre-clinical model of surgically induced cranial bone defect in Wistar rats. The results obtained for cellulose-based nanofiber, cryosponge and hybrid scaffolds will be presented. These were compared with a commercial scaffold used as a positive control. Healing was monitored over different periods up to 12 weeks using clinical monitoring of animals, CT and PET imaging with the ¹⁸FNa radiotracer histology, immunohistochemistry and immunofluorescence.

RESULTS: The results showed that a strong metabolic stimulation induced by the scaffolds, allowing complete healing of the bone defect. Due to their structural constitution, the scaffolds presented different healing profiles.

DISCUSSION & CONCLUSIONS: The results showed that porosity seemed to be an important parameter in the scar stimulation capacity of scaffolds. This work is on-going on a more complex in vivo model of pathological bone regeneration, such as long bone skeleton defect on animal with metabolic disorders.

Keywords: Bone and bone disorders (osteoporosis etc), Imaging - in vivo

PP-138

Design and prototyping of a synthetic eggshell for the ex ovo engineering of frontier models of microvascular physiopathology and advanced therapeutics

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INTRODUCTION: The microvascular network is a target in traditional, antibody-based and small molecule-based drug development for diabetes, retinal disease, virology and cancer research. The chorioallantoic membrane (CAM) model is a valuable tool in this field [1]. In this work, we designed and developed a synthetic eggshell for the engineering of study models ex ovo, to allow fast and versatile assays of the microvasculature in response to injected drugs. We used computational modelling to predict oxygen consumption of an avian embryo at different embryonic incubation days (EID). We then identified a synthetic eggshell substitute material and relevant thickness allowing a physiological oxygenation of the embryo while maintained ex ovo.

METHODS: We modeled the oxygen consumption of a living chicken embryo in its own eggshell, during its development from EID 4 to 14. The computational model was set-up using Comsol Multiphysics. We modeled the egg as a prolate ellipsoid having radius of 1.4 and 1.7cm, using a finite element mesh of 500k tetrahedral elements. We assumed the eggshell as a porous material having a thickness of 365µm, porosity ~0.0248% and tortuosity=1. We assumed the air sac volume ranging from 1.37 to 5.28cm³. For the CAM, i.e. the embryo respiratory organ, we assumed a consuming surface of ~41cm² with a peak oxygen consumption, $V_{max}=2.05 \times 10^{-6} \text{ mol/m}^2\text{s}$, and a consumption rate governed by the Michaelis-Menten kinetics. We assumed three values of PDMS thickness for the eggshell substitute material: 100,365, and 800µm. Finally, we fabricated a prototype of the synthetic eggshell, in which we maintained chick embryos in culture ex ovo.

RESULTS: The simulations predicted an oxygen concentration drop, alongside the eggshell thickness, in the range of 1.5-14% with respect to saturation value (EID4-14). The air sac average oxygen concentration was equal to 8.152mol/m³. In the eggshell substitute made with the PDMS membrane, we predicted a decrease in air sac concentration ranging from 20% to 80%. We fabricated the first prototype of the synthetic eggshell by means of stereolithography and replica molding. The PDMS membrane of 365µm allowed to reach a good viability on the embryos up to EID8 (n=3), allowing for optical accessibility and ease of access of the CAM microvascular network.

DISCUSSION & CONCLUSIONS: Our computational predictions of oxygen drop in the egg were in good agreement with measured values found in literature[2] with a mean error lower than 5%. The eggshell prototype allowed unprecedented versatility and ease of access of the embryo microvascular system.

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REFERENCES: 1.Conci, Adv.Opt.Mat.2022; 2.Ackerman&Rahn,1980

Keywords: In vivo and animal models, Biomaterials

PP-139

Elemental features and ECM remodelling in lung fibrosis pathology: news from the nanoscale

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INTRODUCTION: Idiopathic pulmonary fibrosis (IPF) is a complex lung disease characterised by an exacerbated remodelling of the Extracellular Matrix (ECM). Recent studies have pointed out that trace metals such as Fe and Zn, which display a dysregulated metabolism, are important events during the development of IPF. However, scarce information is known about the link of this event and the ECM remodelling occurring in fibrosis. Synchrotron-based X-Ray Fluorescence (XRF) mapping allowed measuring the elemental distribution of biological samples with sub-micron spatial resolution. Furthermore, macrophages, pivotal leukocytes in lung diseases, are prominent regulators of Fe metabolism. Recent studies have shown macrophage-to-fibroblast paracrine crosstalk contribute to the development of fibrosis. Accordingly, the aim of this study is to explore IPF-relevant elemental features in lung human samples by synchrotron-based XRF mapping and to evaluate their link to tissue remodelling mediated by macrophages in lung fibrosis.

METHODS: Distal lung from a healthy and an IPF individual were fixated and dehydrated in serial acetone solutions. Afterwards, samples were embedded in epoxy-like resin, cured at 65 ° C during 72h and sectioned at 1 µm thickness. The slices were mounted on Si3N4 membranes. XRF mapping was performed at the NanoMAX beamline (MAX IV) using a photon energy of 10 keV at 100 nm steps on two different areas for each healthy and diseased sample. Additionally, adjacent slices from each scanned samples were placed on glass slides and stained for Movat Pentachrome staining and pan-macrophages markers, respectively.

RESULTS: IPF samples displayed increased number of anthracotic bodies that showed a heterogeneous coating of several trace metals such as Ti, Cu, Fe, Cr and Ni. IPF showed a cluster accumulation of Ca in the whole parenchyma, while healthy tissue showed an accumulation of Zn only within the nuclei. By correlation of histologic and XRF images, IPF showed S and Fe accumulation within cytoplasmic granule-like structures (<1 µm). Interestingly, areas of increased cells with Fe-S accumulated were surrounded with extensive remodelling of fibrillary collagens.

DISCUSSION & CONCLUSIONS: To our knowledge, this is the first study on IPF human samples giving insights into elemental markers of IPF pathophysiology. Additionally, this data points out a potential link of the elemental metabolism carried out by macrophage and the tissue remodelling characteristic of IPF. The sub-cellular resolution of XRF and the correlation to histological imaging herein opens an avenue to study tissue remodelling from diseases that are accompanied with an elemental dysregulation of the tissue.

Keywords: Imaging - advanced, Immunity / immunomodulation / macrophage

PP-140

Application of Large Area Mapping AFM for Automated Structural and Mechanical Analysis of Developing Cells and Tissues

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INTRODUCTION: AFM can be successfully applied for comprehensive nano-mechanical characterization of single molecules, cells and tissues, under near physiological conditions [1]. Currently, the trend is to extend this by studying the mechanobiology of living cells while evaluating their structure and the interaction with their cell culture substrates [2,3]. It is interesting to understand how cell behaviour is driven by the cytoskeletal dynamics and cell mechanics in typical cell culture scaffold scenarios. We will introduce the concept of automated large area multiparametric characterization of densely packed cell layers and highly corrugated tissue samples.

METHODS: We applied high-speed AFM, with a temporal resolution on the second to millisecond scale to resolve dynamic processes such as the collagen fibrillogenesis and cytoskeletal dynamics in living cells. As a tool for analyzing the complex cellular mechanobiology, we went beyond purely elastic models, and performed sine oscillations (up to 1 kHz, amplitude 5-60 nm) in Z while in contact with the surface to probe the frequency-dependent response of living fibroblasts.

RESULTS: We will provide insight into the structural formation of collagen type I, emphasizing the intermediate steps in the process [4]. We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be associated with spatially resolved cytoskeletal reorganization events. We will further discuss how to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus (E' , E'') distribution in living fibroblast cells. In the past, investigating large and rough samples such as tissues and hydrogels using AFM was challenging due to the limited z-axis of the AFM. Using osteoarthritic cartilage as an example, we will demonstrate how a newly developed stage enables multi-region AFM probing over a large, rough sample area while providing additional correlative optical data sets [5].

DISCUSSION & CONCLUSIONS: We will discuss how these developments, in combination with advanced optical microscopy techniques, can overcome the inherent drawbacks of traditional AFM systems for characterizing challenging biological samples.

ACKNOWLEDGEMENTS:

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Imaging - advanced

PP-141

Tissue engineered tendon: A 'universal' (multi-platform) 3D printed tensile bioreactor chamber for cyclic strain enables multimodal analysis of collagen isoform composition and fibril architecture

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INTRODUCTION: Tendon is predominantly composed of aligned type I collagen, but additional isoforms are known to influence fibril architecture and contribute to overall biomechanical performance. In this study, human mesenchymal stem cells (MSCs) were cultured under tenogenic conditions in a fibrin scaffold with 3-10% cyclic strain for 3 weeks, and a comprehensive multimodal analysis comprising qPCR, Western blotting, histology, mechanical testing, fluorescent probe CLSM, TEM and label-free second-harmonic imaging was performed. We designed and 3D printed a 'Universal' multi-well tensile bioreactor chamber that attaches to a range of commonly used actuator platforms (e.g. EBERS-TC3, CellScale MCT6 and TA ElectroForce) in order to increase our experimental capabilities and enable better direct comparison between studies.

METHODS: We designed a bioreactor chamber with six independent wells that was 3D printed in PLA [1]. Tissue-engineered tendons were generated from hMSCs in a fibrin hydrogel scaffold with 0, 3%, 5% or 10% cyclic strain at 0.5Hz for 3 weeks, and a comprehensive multimodal analysis was performed [2].

RESULTS: Molecular data indicated complex transcriptional and translational regulation of collagen isoforms I, II, III, V, XI, XII and XIV in response to cyclic strain. Isoforms (XII and XIV) associated with embryonic tenogenesis were deposited in the formation of neo-tendons from hMSCs, suggesting that these engineered tendons form through some recapitulation of a developmental pathway. Tendons cultured with 3% strain had the smallest median fibril diameter but highest resistance to stress, whilst at 10% strain tendons had the highest median fibril diameter and the highest rate of stress relaxation. Fluorescent probe images correlated increasing cyclic strain with increased fibril alignment from 40% (static strain) to 61.5% alignment (10% cyclic strain).

DISCUSSION & CONCLUSIONS: Correlating the abundance of less well-studied collagen isoforms with fibrillogenesis and biomechanical changes is a promising method for characterising optimised bioengineered tendon grafts [2]. The widespread availability of 3D printing now provides an opportunity to develop an open source 'universal' bioreactor chamber that can be coupled to a wide range of commonly used linear actuator platforms, resulting in a greater comparability between research groups [1].

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), 3D printing and bioprinting

PP-142

Extracellular matrix coding and reading: alterations under compromised conditions and consequences for cell behaviour in tissue regeneration

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INTRODUCTION: Natural aging and obesity are associated with several risk factors that may result in impaired bone regeneration, including bone marrow (BM) adipogenesis, an altered immune cell composition and decreased cellular mechano-sensitivity. Clinical studies showed that marrow adipogenesis correlates with decreasing bone mineral density and an increasing risk for fractures and healing complications [1,2]. On the cellular level, this condition is presented by a reduced osteogenic and increased adipogenic differentiation potential, decreased proliferation rates and enhanced senescence of BM mesenchymal stromal cells (MSCs) [3-6]. The overarching aim of this project is to understand if and how information encoded in the ECM influences cell function in the context of bone regeneration under compromised conditions related to aging.

METHODS: Therefore, we examined whether ECM deposited by MSCs from compromised niches might contain a compromised “matrix-code”.

To study the influence of the adipocyte secretome on ECM secretion and function of MSCs, we created a biomaterial-based 3D in vitro micro tissue model using a MSC/adipocyte co-culture-system and adipogenically-conditioned medium. Differences in the amount, distribution and structural organisation of the ECM were analysed by immunofluorescence staining. Next, we want to complement these observations by assessing mechanical tension, stiffness and viscoelasticity of grown microtissues.

RESULTS: Adipogenically-conditioned medium results in reduced collagen type I secretion, while collagens (type I and IV), as well as fibronectin are less oriented.

DISCUSSION & CONCLUSIONS: Cultivation of hMSCs in adipogenically-conditioned medium changes ECM composition in 3D. Thus, the adipogenic secretome might impact MSC ECM secretion and ECM deposited by MSCs from a compromised niche, might contain a compromised “matrix-code”.

ACKNOWLEDGEMENTS:

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Bone and bone disorders (osteoporosis etc)

PP-143

Best practices for characterising the Mechanical Properties of Native Tissues and In Vivo Mechanical Stimulation

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INTRODUCTION: Understanding the mechanical response of native tissues is paramount to the development of biomaterials that can undertake, augment or completely replace the natural function as a medical device. A variety of mechanical characterization methods are used among researchers to characterize stiffness, modulus, and viscoelastic response of synthetic as well as natural biomaterials. Mechanical stimulation techniques are also increasingly used to understand the biomechanical response of healthy, diseased, or treated tissues. In this talk, best practices for mechanical characterization and a variety of in-vivo mechanical stimulation methods will be presented.

METHODS: Dynamic Mechanical Analysis

Quasi-static Modulus Testing

Mechanical Stimulation

Fatigue Testing

RESULTS: To be decided

DISCUSSION & CONCLUSIONS: To be decided

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Biomaterials

PP-144

Healing-promoting effects of mesenchymal stem cells

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INTRODUCTION: Mesenchymal stem cells (MSCs) are considered pro-regenerative but the exact mechanism of action of these properties remains vague. The hypothesis that MSC directly replace the damaged tissue has been disproven and has been superseded by the idea that MSC stimulate innate regeneration via paracrine regulation of crucial physiological processes (mainly inflammation).

METHODS: Canine MSCs from adipose tissue (ASC) or bone marrow (BMSC) were isolated by standard methods and characterized by FCM. The conditioned media (CM) from MSCs were prepared in serum-free media (for 24 - 96 h), 0.22µm filtrated, and stored at -80 °C. 3T3 fibroblasts were cultured in 10% DMEM. Cytokine composition in CM was measured by canine cytokine array (Raybiotech), and VEGF was quantified by ELISA. TGFβ pathway activation was measured in a 3T3 stable reporter cells transfected with the plasmids from Promega. Scratch assay was performed using Incucyte S3.

RESULTS: Canine ASC and BMSC were able to survive in serum-free basal media for 96 hours, but the prolonged incubation led to the detachment of some cells. For most experiments, CM obtained after 24, 48, and 72 h were used. CM aspirated after 72 h was usually the most potent. The CM from canine MSC was able to potentiate the migration of 3T3 fibroblasts. The wound closure in the scratch assay was significantly higher (31.8 – 42.0 %) in CM-treated fibroblasts than in the serum-free control (15.2 %). There was no significant difference between ASC and BMSC. Interestingly, a significant difference was detected in VEGF content in CM from these two cell types (330 vs 1068 pg/ml) and a slight difference was also in their ability to activate the TGFβ pathway in 3T3 fibroblasts. In both assays, the BMSC performed better than ASC. But in general, both BMSC and ASC were able to produce VEGF and TGFβ. Moreover, the cytokine array showed the presence of other potentially pro-regenerative molecules present in the CM from canine MSC, such as MCP-1, while proinflammatory cytokines retarding the wound healing such as TNFα or IL-8 have been absent or present in very low concentrations, respectively.

DISCUSSION & CONCLUSIONS: The effects of MSC on immune cells have been studied extensively for several years and it can be one of the ways how MSC positively affects regeneration and wound healing. In our work, we have shown that CM from canine MSCs can also positively affect other physiological processes involved in wound healing, cell migration, immune cell recruitment, and angiogenesis.

Keywords: Stem cells - general, Wound healing

PP-145

Investigating the complement system effect on Mesenchymal stromal cells and using a biomaterial delivery tool for improving the bone and cartilage regeneration

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INTRODUCTION: Mesenchymal stromal cells (MSCs) are considered an ideal cellular therapy for several degenerative conditions of bone and cartilage because of their ability to regenerate and immunomodulation¹. However, these capacities can be altered by the degenerative microenvironment². In osteoarthritis and rheumatoid arthritis, complement factors such as C3a are overly expressed in the diseased tissues³. In contrast, low C3a has been found to be associated with defective healing of bone fractures⁴.

The primary aim of the study is to investigate how the complement system, particularly C3a and C5a, can affect MSC regenerative functions. The second aim is to assess if capsules fabricated using the Layer-by-Layer assembly method⁵ can help deliver these complement factors and potentially their antagonists to modify MSC reparatory functions.

METHODS: The human adipose MSCs were cultured in vitro and characterized using flow cytometry. The XTT and calcium assays were used to assess MSC proliferation and osteogenic differentiation, respectively. CaCO₃-templated hyaluronan/polylysine capsules were tested as a delivery tool for complement protein uptake. The uptake capacity of capsules was investigated by incubating capsules with complement Factor B protein and complement factor C3a, followed by ELISA. The visualisation of MSCs and capsules was done using fluorescence microscopy.

RESULTS: The cultured MSCs maintained their surface phenotype throughout the study. The effect of C3a on MSC proliferation was minimum, but C5a caused a reduction in MSC proliferation. C3a induced the calcium deposition of MSCs cultured in osteogenic media, unlike C5a, which did not change the calcium deposition by MSCs. The protein uploading kinetics by capsules was tested using 1.4x10⁵ capsules with 60ng of factor B protein and showed a maximum protein upload after 24 hrs. The temperature range of 4-20°C did not affect protein uptake. However, a dynamic loading has led to almost complete C3a protein uptake within 4 hours. Imaging data indicated the capsule adhesion to cells.

DISCUSSION & CONCLUSIONS: This study demonstrates a new biological link of the complement system to bone regeneration via its effect on human MSC functions. Ongoing work aims to test the complement system effect on MSC chondrogenic and immunomodulatory potentials.

The data also introduces the CaCO₃-templated hyaluronan/polylysine capsules as a new tool to deliver or target the complement system aiming to improve the bone and cartilage regenerative potential of MSCs.

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Keywords: Cell therapy, Drug delivery

PP-146

Induction of PLXNA4 gene during neural differentiation in human umbilical cord-derived mesenchymal stem cells by low intensity sub-sonic vibration

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INTRODUCTION: Human umbilical cord-derived mesenchymal stem cells (hUC-MSC) are one type of mesenchymal stem cells and more primitive than other MSCs. In this study, we identify novel genes and signal-activating proteins involved in the neural differentiation of hUC-MSCs induced by Low Intensity Sub-Sonic Vibration (LISSV). In the present study, we intend to investigate key genes involved in this differentiation by sonic vibration only through RNA sequencing based on next-generation sequencing.

METHODS: RNA sequencing assay was used to find genes involved in the differentiation process by LISSV. The changes of hUC-MSCs caused by LISSV were confirmed by PLXNA4 overexpression, and gene knockdown through small interfering RNA experiments.

RESULTS: The six genes were increased among genes related to neurons and the nervous system. One of them, the PLXNA4 gene, is known to play a role as a guide for axons in the development of the nervous system. When PLXNA4 recombinant protein was added, neuron-related genes were increased. In the PLXNA4 gene knockdown experiment, the expression of neuron-related genes was not changed by LISSV exposure. The PLXNA4 gene is activated by sema family ligands. The SEMA3A gene was increased by LISSV, and its downstream signaling molecule, FYN, was also activated.

DISCUSSION & CONCLUSIONS: We reported neuronal differentiation of hUC-MSCs caused by LISSV alone, one of the mechanical stimuli. After 4–5 days, the shape of hUC-MSCs modified to neuron-like and neuron-related genes were expressed. This neuronal differentiation was activated through the Nrp/plxna4 receptor complex with the SEMA3A-dependent signaling mechanism. Discovering the specific mechanism that induces neural differentiation in hUC-MSCs via LISSV and applying it to neurodegenerative disorders will be very useful in stem cell therapy.. We suggest that the PLXNA4 gene plays an important role in hUC-MSC neuronal differentiation through exposure to LISSV. The differentiation process depends on SEMA3A-PLXNA4 dependent FYN activation in hUC-MSCs.

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Keywords: Stem cells - general, Biomechanics / biophysical stimuli and mechanotransduction

PP-147

Investigating the Role of NF- κ B in Tendinopathy Using Equine Embryonic Stem Cells

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INTRODUCTION: Tendon injuries are a major cause of musculoskeletal morbidity in both humans and horses. Post-injury, the healing response is inadequate leading to the formation of scar tissue which is prone to re-injury. Despite evidence suggesting insufficient resolution of inflammation leads to fibrotic healing, our understanding of the inflammatory pathways implicated in tendinopathy is lacking. Previously, IL-1 β was shown to have adverse effects on equine adult tenocytes, but equine embryonic stem cell (ESC) derived tenocytes were unaffected¹.

METHODS: Equine adult and ESC-tenocytes were cultured in three-dimensional (3-D) collagen gels under control or IL-1 β conditions for 14 days and transcriptomic analysis performed. Equine adult and ESC-tenocytes were also stimulated with IFN- γ , TNF α and IL-1 β or NF- κ B activator/inhibitor pharmaceuticals prior to analysis of gene expression, immunofluorescence to determine inflammatory pathway activation and 3-D collagen gel contraction. Additionally, adult tenocytes were stimulated with inflammatory cytokines alone or with ESC-tenocyte conditioned media and any protective effects on gene expression determined.

RESULTS: RNA sequencing analysis demonstrated adult tenocytes exhibit 2517 differentially expressed (DE) genes, whereas ESC-tenocytes had zero DE genes following IL-1 β stimulation. Enrichment analysis revealed NF- κ B signalling as the only inflammatory pathway activated. Stimulation of adult tenocytes with IFN- γ , TNF α and IL-1 β resulted in translocation of NF- κ B to the nucleus and DNA binding, which led to significant changes to tendon-associated gene expression and impaired the cells' ability to contract a 3-D collagen gel. ESC-tenocytes were unaffected by cytokine stimulation and no NF- κ B activation was observed. However, NF- κ B could be activated in ESC-tenocytes using pharmaceuticals and this resulted in adverse changes to tendon-associated gene expression. Pharmaceutical inhibition of NF- κ B inhibited nuclear translocation of NF- κ B in adult tenocytes following short term inflammatory stimulation but failed to prevent changes to tendon-associated gene expression following more sustained inflammatory stimulation. Interestingly, ESC-tenocyte conditioned media was able to rescue the adverse effects a combination of inflammatory cytokines has upon tendon-associated gene expression in adult tenocytes.

DISCUSSION & CONCLUSIONS: We demonstrate IL-1 β , TNF α and IFN- γ work synergistically to induce greater detrimental consequences for adult tendon regeneration than when used individually. However, ESC-tenocytes appear to be unaffected by inflammatory stimulation. Our results suggest that NF- κ B may be responsible for the adverse downstream consequences of these inflammatory cytokines. Understanding the mechanisms by which ESC-tenocytes are unaffected by inflammatory stimulation may enable the identification of novel interventions for adult tendon injuries which minimise scar-tissue formation.

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Keywords: Embryonic stem cells, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-148

Improving analysis of human induced mesenchymal stromal cells during chondrogenic differentiation through encapsulation in single cell microgels

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INTRODUCTION: Due to the low regenerative capabilities of chondrocytes, *in vitro* generation of durable, large-scale (10+ cm²), high-quality neocartilage for implantation into osteoarthritic (OA) joints is urgently needed. Generation of *de novo* cartilage from cell types such as human induced mesenchymal stromal cells (hiMSCs) are attractive due to their benefits as a stable and sustainable cell source. However, the quality of neocartilage generated by hiMSCs has lower quality when compared to primary chondrocytes. To improve hiMSC chondrogenesis, unbiased characterization of the differentiation is needed. This is made difficult by the harsh methods typically used to remove cartilage extra-cellular matrix (ECM) deposited during differentiation. By encapsulating hiMSCs in single cell microgels, the deposition of such dense intercellular matrix is avoided. This allows more gentle processing and therefore reduces stress induced bias for more accurate characterization.

METHODS: hiMSCs were generated [1] and encapsulated as single cells in 5% Dex-TA using two distinct microfluidic chips (one for droplet generation and another for delayed gelation)[2, 3]. Encapsulated hiMSCs (sciMSCs) were cultured in trans-well plates and refreshed bi-weekly with hiMSC chondrogenic differentiation medium. Viability of the encapsulated cells was determined, deposition of chondrogenic markers was investigated using immunofluorescent staining, and gene expression was analyzed by RT-qPCR after two weeks of chondrogenesis.

RESULTS: The microgels remained stable and supported hiMSC survival (>80%) throughout the 2-week chondrogenic differentiation. Gene expression analyses showed increased expression of ECM genes such as *COL2A1* and *ACAN* confirming chondrogenic differentiation of hiMSCs. In line with this, immunostaining showed deposition of chondrogenic markers (COL2, ACAN, and COL6) after 14 days of differentiation.

DISCUSSION & CONCLUSIONS: Gene and protein expression show that hiMSCs readily deposit cartilage ECM within sciMSCs indicating the generation of chondrocyte-like cells similar to differentiation of bone marrow derived MSCs. Moving forward, we will now utilize transcriptome-wide single cell analysis to identify key cell fate decisions that can be controlled to improve hiMSC chondrogenic potential.

ACKNOWLEDGEMENTS: Financially supported by the Dutch Research Council (NWO, OCENW.GROOT.2019.079)

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Keywords: Multipotent (mesenchymal) stem cells, Cartilage / joint and arthritic conditions

PP-149

Pectin microneedle array patch for the delivery of therapeutic cells for brain injury

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INTRODUCTION: Animal studies investigating brain injury have demonstrated improved neurological outcomes following mesenchymal stromal cell (MSC) transplantation¹, however successful delivery of these cells is limited by the delivery route. Local delivery overcomes the large loss of therapeutic cells observed following intravenous delivery, therefore we hypothesise that delivering cells directly to the injury site using a pectin microneedle array patch (MAP) will result in higher retention of cells, therefore improving therapeutic outcomes. Pectin has previously demonstrated high biocompatibility.

METHODS: MAPs with 100 obelisk projections, each with a height of 1000 μm , were made using a two-step casting process. The needle layer was manufactured using a pectin-based formulation, loaded into a PDMS mould. A backing layer, made using sodium carboxymethyl cellulose, water, and glycerol, was added to aid manipulation and placement of the MAP. MAPs were stored in a desiccator and left to dry at room temperature prior to demoulding.

Environmental scanning electron microscopy (ESEM) facilitated visual inspection to confirm that the drying and demoulding processes are acceptable. Fracture force was assessed using a texture analyser to understand the maximal force that can be applied before causing irreversible damage to the MAP. Cytotoxicity testing on pectin MAPs was carried out following the ISO 10993-5, using the direct and elution method. SH-SY5Y human neuroblastoma cells were used.

0.9% agarose gel was used to simulate brain tissue to evaluate microneedle insertion and cell delivery using 3T3 mouse fibroblasts, before moving onto human MSCs. Cells were seeded onto the microarrays. The migration and viability of the cells was then visualised using confocal microscopy after 3 hours and 24 hours.

RESULTS: ESEM demonstrated that MNs remain intact after being demoulded with well-defined geometry. Fracture force was measured as 0.22 N/microneedle, favouring the successful insertion into ultrasoft brain tissue. Cytotoxicity testing demonstrated the pectin MAPs were not toxic according to the ISO guidance.

Microscopy demonstrated microneedles successfully inserted into 0.9% agarose, forming distinct channels. Both the 3T3 cells and humans MSCs successfully migrated into the channels formed and were retained for 24-hours with high viability.

DISCUSSION & CONCLUSIONS: Pectin MAPs have demonstrated appropriate mechanical and physical properties for insertion into the brain. Furthermore, preliminary cell studies demonstrate the microneedle channels formed enable therapeutic cell retention, offering an alternative delivery platform to enhance therapeutic outcomes in the treatment of brain injury.

ACKNOWLEDGEMENTS:

REFERENCES: (1) Volkman et al., Stem cells, volume 35, 1867-1880, 2017

Keywords: Multipotent (mesenchymal) stem cells, Additive manufacturing

PP-150

Ovine Adipose derived Stem Cells differently respond to distinct teno-inductive *in vitro* stimuli

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INTRODUCTION: Adipose derived stem cells (ADSCs) are alternative stem cell sources in regenerative medicine for tendon healing [1]. Despite their applicative increased attention in tendon engineering strategies, the mechanisms promoting tendon healing, both *in vitro* and *in vivo*, are barely understood. Since there are only few and fragmentary research on ovine ADSCs, this research aims to investigate their properties and *in vitro* tenogenic differentiation for a potential use in tendon regeneration, as sheep is a high translational animal model for this type of pathologies.

METHODS: Ovine ADSCs were isolated from the tail region according to FAT-STEM laboratories [2] and characterized by immunocytochemistry (ICC) for mesenchymal (Vimentin) and stemness (SOX2, NANOG) markers. MTT and Beta-galactosidase assay were performed to evaluate proliferation and cell senescence during expansion. ADSC's teno-differentiation potential was compared by co-culturing ADSCs with fetal tendon explants (FTE) or by engineering them on tendon-mimetic PLGA scaffolds [3] up to 14 days of culture. ADSCs tenogenic differentiation was then evaluated by morphological, molecular (qRT-PCR), and biochemical (Western Blot) approaches.

RESULTS: The isolated ADSCs exhibited mesenchymal phenotype with positive expression of Vimentin and CD44 (82% and 80%, respectively), and were negative for hematopoietic CD45 marker. ADSCs showed nuclear positivity for SOX2 ($\cong$ 56%) and NANOG ($\cong$ 67%), high proliferation capacity without senescence signaling during expansion until passage 6. Interestingly, ADSCs behaved differently on both teno-inductive strategies. ADSCs when co-cultured with FTE, did not shown tenomodulin (TNMD) a mature tendon marker, gene and protein expression. However, they exhibited chondrocyte-like morphology, upregulation of chondrogenic genes (*SOX9*, *COL2*, *ACAN*) and Alcian Blue positivity. Conversely, ADSCs seeded on scaffolds were able to teno-differentiate, acquiring a spindle tenocyte-like shape supported by TNMD protein expression already at 48h ($p < 0.05$ vs. ADSCs 48h) with significant increase at 14 days of culture ($p < 0.05$ vs. ADSCs + fleece 48h).

DISCUSSION & CONCLUSIONS: Data demonstrate that ovine ADSCs can be committed to different mesenchymal-related lineages by exposure to specific teno-inductive signals. ADSCs were pre-committed toward chondrogenic phenotype under FTE released factors, though under tendon mimetic scaffold topological stimulus, underwent toward tenogenic lineage. Therefore, before using ADSCs *in vivo*, for tendon regeneration more insights are requested to predict their commitment in the host tissue.

ACKNOWLEDGEMENTS: This research is part of the P4FIT project ESR5, funded under the H2020MSCA-ITN-EJD-P4 FIT—Grant Agreement ID: 955685.

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Keywords: Differentiation, Stem cells - general

PP-152

Evaluation of the senescence process of human mesenchymal stromal cells from older patients in in vitro culture

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INTRODUCTION: Development of regenerative medicine forces the search for new sources of cells for the reconstruction of tissues and organs. One of the most popular cells in regenerative medicine are mesenchymal stromal cells (MSCs) due to their unique properties. Good source of MSCs is adipose tissue because of the high availability and easy, minimally invasive access. Despite the enormous regenerative potential, the use of MSCs in clinical practice may also be associated with potential problems that limit their application. Long-term culture of MSCs allows to obtain a large number of cells, but may also lead to reduction of their regenerative potential as a result of cellular senescence. The aim of the study was to evaluate selected molecular markers of the cellular senescence in human AD-MSCs from older patients in a long-term in vitro culture.

METHODS: Human AD-MSCs were isolated from lipoaspirate obtained by liposuction from oncological patients during radical cystectomy. Cells were cultured up to the 10th passage using two serum free culture media (MI or MII). Cells from the 2nd, 6th and 10th passage were used for the research. The immunophenotype of AD-MSCs was confirmed by flow cytometry. RNA was isolated from AD-MSCs and used for cDNA synthesis. The qPCR reaction was performed using a set of 96-well plates dedicated to the analysis of 89 genes related to human aging.

RESULTS: Long-term in vitro culture of AD-MSCs in both media alters cells morphology and expression of genes associated with aging. Distinct morphology changes like enlargement, irregular edges and numerous granules were seen before 10th passage. Flow cytometry analysis of AD-MSCs from selected passages confirmed immunophenotype typical for MSCs. Cells cultured in MI showed changed expression of 11 genes. Expression of 8 genes was increased (ANXA5, FBXL16, PANX1, SIRT6, TFB1M, TPP1, WRN, ZMPSTE24), and decreased expression was observed for 3 genes (CDKN1C, FOXO1, VWA5A). Cells cultured in MII showed changed expression of 7 genes. 5 of them were increased (ANXA3, ANXA5, RNF144B, S100A9, ZMPSTE24) and 2 genes were decreased (C3 and CDKN1C). These genes were related to the control of the cell cycle, stimulation of proliferation, induction of cell death, secretory functions and intercellular signaling.

DISCUSSION & CONCLUSIONS: Research showed that AD-MSCs can be successfully isolated from adipose tissue of older patients and cultured up to the 6th passage with no significant signs of cellular senescence. Both tested culture media can be used in the in vitro culture of AD-MSCs.

Keywords: Multipotent (mesenchymal) stem cells, In vitro microenvironments

PP-153

Miniaturizing culture ware with SH-SY5Y cell line

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INTRODUCTION: Traditionally, in vitro brain models include primary neuronal cultures from rats and mice, neuroblastoma cell lines including rat B35 and mouse Neuro-2A cells, rat PC12 cells cultured in static 2D culture ware. Unfortunately, these models lack sufficient recapitulation of in vivo-like features of the human brain. Hence, Brain-on-Chips (BoCs), incorporating microfluidics and 3D scaffolds, became popular. However, scaling-down culture volumes in BoCs remains a challenge. Here, we demonstrate how miniaturizing affects SH-SY5Y human neuroblastoma cell line cultures.

METHODS: We compare SH-SY5Y cells seeded on a 6-well plate (Thermo Scientific Non-Treated-150239) with cells on μ -Slide Angiogenesis Glass Bottom (IBIDI-81507). Differentiation of SH-SY5Y cells relies on serum deprivation; the addition of retinoic acid and brain-derived neurotrophic factor. The cell medium is refreshed on day 1, 3, and 6 and then every second day until day 21. Optical images (Leica DMI8 fluorescent microscope) were taken at day 1, 3, 6, 10 and 21. Cells were stained on day 21 with anti-MAP2 (1:1000; ab32454, Abcam), and anti-SMI312 (1:500; 837904, Biolegend), which are neuronal markers expressed in differentiated neurons.

RESULTS: Phase-contrast microscopy images showed that cells populated more and differentiated faster in a larger volume culture ware, i.e. 6-well plates compared to the smaller volume IBIDI slides. Also, most of the cells have been lost in the IBIDI slide after washing and many protrusions of the cells were lost during the fixation process before the immunostaining on day 21. However, in both types of culture ware differentiated neurons were observed as indicated by the presence of anti-MAP2 and anti-SMI312 staining.

DISCUSSION & CONCLUSIONS: We tested SH-SY5Y cells in two types of culture ware that were mainly distinguished by their volumes but also by their material (tissue culture plastic versus glass), but regardless of the choice, cells survived and differentiated. Though, it was observed that cells in the 6-well plate were less affected by media exchange and washing steps of the fixation protocol and initially continued to proliferate and hence connected by shorter protrusions already into a network. On the other hand, besides the material, most of the cells in the small volume IBIDI slide were exposed to the pipetting pressure and inevitably such forces are also strong enough to break up connections between the cells. Staining results still support that static small volume IBIDI slide culture ware supports successful differentiation but potentially could be improved if pipetting issues can be resolved by adding microfluidic fluid exchange in appropriate BoC designs.

Keywords: Differentiation, In vitro microenvironments

PP-154

microRNAs during osteogenic differentiation: bipolarity or target preference?

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INTRODUCTION: Bone metabolism is highly regulated and recent studies showed that microRNAs (miR) can contribute to the deregulation of proteins needed to maintain healthy bone structures. Seeliger et al. already identify five miR (miR-21, miR-23a, miR-24, miR-100, and miR-125b) associated with osteoporotic fractures. Overexpression of miR-125b is well-known to enhance osteoporosis and regulate osteogenic differentiation of mesenchymal stem cells (hMSCs) via several pathways. Here we evaluated the involvement of the pro-osteoporotic miR-125b in BMP pathway as well as in mineralization.

METHODS: hMSCs (n=5) were transfected with mimic of miR-125b or scrambled miR (negative control) combined or not with osteogenic differentiation. Two different protocols of differentiation were tested, one using dexamethasone and another using calcium saturated medium. Mineralization was assessed by Alizarin Red staining, microRNA expression by qPCR and proteins by western blotting.

RESULTS: Mimicking of miR-125b in hMSCs cultured in basic medium is associated with a decrease of miR-100-5p and miR-21 expression, and correlated with an increase of RUNX2 and BMPR2 expression.

Exposure to dexamethasone or calcium-enriched medium increased mineralization of hMSCs compared to basic medium and is accompanied by a decrease of miR-125b expression. Interestingly this seems to be correlated with increased RUNX2, BMPR2 and SMAD4 expression measured at day 7.

We observed that hMSCs transfected with miR-125b mimic and stimulated with dexamethasone presented a higher mineralization compared to cells transfected with scrambled miR and this observation seems to be correlated with a higher expression of RUNX2, SMAD4 and BMPR2. Interestingly cells treated with calcium-enriched medium and transfected with mimic of miR-125b showed a decrease in mineralization compared to scrambled miR. This result is supported by a decrease of RUNX2 and BMPR2 in cells transfected with the mimic. Accordingly, inhibition of miR-125b in calcium-treated cells increased mineralization compared to scrambled miR and seems to upregulate BMPR2 and RUNX2 expression.

DISCUSSION & CONCLUSIONS: We highlighted that miR-125b differentially modulates mineralization of hMSCs in dexamethasone and calcium-induced osteogenic differentiation. This dissimilarity can be explained by the difference in the pathways stimulated by the two treatments. Therefore, miR-125b affinity for targets implicated in bone remodeling changes and induces an opposite final physiological effect. Moreover, the down-regulation of miR-100-5p, another pro-osteoporotic miR, following the mimicking of miR-125b can also impact targets of interest. Thus, dynamics of miRs during osteogenic differentiation needs to be studied. With this work we slightly open the door to possible therapeutic approaches for bone diseases such as osteoporosis.

Keywords: Bone and bone disorders (osteoporosis etc), Gene therapy

PP-155

Light Driven Control of Chondrogenic hPSC Differentiation in 3D

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INTRODUCTION: Chondrogenesis protocols that aim to drive hPSC differentiation to articular chondroprogenitors rely initially on bone morphogenetic protein (BMP), and later, on transforming growth factor beta (TGF β). Protocols inefficiently generate a stable phenotype due to batch-variation of growth factors which provide limited control over cell signalling. To enhance precision, optogenetics has been applied to such protocols and has provided blue light-driven control over BMP signalling to replace the growth factor in 2D.¹ However, translation of the optogenetic BMP (optoBMP) system to 3D, and light-driven control of additional signalling pathways, are necessary to harness the potential of optogenetics in cartilage tissue engineering.

METHODS: To assess optoBMP system activation in 3D, immortalised chondrogenic cells containing the optoBMP system (optoBMP-TC28a2) were encapsulated in 2% alginate. OptoBMP-TC28a2 laden hydrogels were stimulated with 470nm light for 4 hours and gene expression quantified afterwards by RT-qPCR. OptoBMP-hESCs were differentiated to chondroprogenitors over 11 days with blue light irradiation replacing the usual BMP growth factor addition on Days 7 to 11. At Day 11, chondroprogenitors were transitioned to 3D culture by encapsulation in 2% alginate. Cell viability and gene expression were determined at Days 3, 7 and 14 following encapsulation by RT-qPCR.

RESULTS: Alginate encapsulated optoBMP-TC28a2 cells had increased chondrogenic (*COL2A1*) and BMP response (*ID1*, *ID2*) gene expression following light irradiation compared to optoBMP-TC28a2 irradiated in 2D. After 7 days in alginate, optoBMP-hESC derived chondroprogenitors continued to express chondrogenic genes (eg. *COL2A1*, *SOX9*). When transitioned to 3D hydrogel culture, chondroprogenitors derived from light-stimulated differentiation protocols had higher expression of chondrogenic *SOX9*, *SOX5* and *RUNX2* compared to chondroprogenitors derived from BMP growth factor stimulated protocols.

DISCUSSION & CONCLUSIONS: Results from irradiation of optoTC28a2-laden alginate are evidence that optoBMP system activation is achieved when cells are in a 3D environment. Furthermore, the ability of light-stimulated chondroprogenitors to retain chondrogenic potential in a 3D biomaterial system has been demonstrated. Together, these findings provide motivation for application of biofabrication technologies, such as light-assisted 3D bioprinting, to generate chondrogenic tissue analogues in which cell signalling pathways of maturing chondroprogenitors can be dynamically manipulated with light. In parallel, progress has been made towards development of an orthogonal red light-sensitive optoTGF β system with the goal that, in future, precise spatial and temporal probing of multiple signalling pathways with different light wavelengths could provide fine-tuned control over cellular phenotype in 3D.

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Keywords: Cartilage / joint and arthritic conditions, Biomaterials

PP-156

Comparison of differentiation possibilities of Adipose Tissue-derived Mesenchymal Stromal/Stem Cells and progenitor Buccal Epithelial Cells towards porcine Urothelial Cells

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INTRODUCTION: Construction of many tissues and organs de novo requires the use of external epithelial cell sources. In the present study we tested whether Adipose Tissue-derived Mesenchymal Stromal/Stem Cells (AD-MSCs) and progenitor Buccal Epithelial Cells (BECs) are able to differentiate into Urothelial Cells (UCs).

METHODS: UCs, AD-MSCs and BECs were isolated from tissues harvested from domestic pigs during planned economic slaughters and cultured to the 3rd passage. Simultaneously, the conditioned medium (CM) was collected from confluent UCs cultures between the 1st–the 3rd passage (n=5). To induce differentiation 70-95% confluent AD-MSCs and BECs cultures after the 3rd passage were exposed to prepared CM for 14 days and analysed on days: 0, 7th and the 14th of differentiation process. AD-MSCs, BECs and UCs cultured in their standard media were used as controls. To characterize and compare the differentiation effect of CM, the immunofluorescent stains and flow cytometry analyses for ck7, ck14 and upIII were performed. Additionally, the expression of ck8, ck13, ck14, ck19, uroplakins (1a, 1b, 3a, 3b), p63, integrin β 1, cd90 and cd105 was analysed by Real-Time PCR.

RESULTS: Immunofluorescent analysis demonstrated: the presence of ck7 in UCs only and the presence of ck14 in UCs and BECs. The addition of CM had no effect on ck7 expression in AD-MSCs and BECs and ck14 expression in AD-MSCs. UpIII was expressed by UCs and BECs. There was higher expression of upIII in BECs cultured with CM ($p < 0,0001$). Flow cytometry analyses have shown that CM has no effect on ck7, ck14 and upIII expression. Real-Time PCR confirmed higher expression of ck8, ck14 and up1b in the case of BECs from cultures established with the addition of CM. CM did not induce any other changes in expression of tested genes.

DISCUSSION & CONCLUSIONS: It was demonstrated that BECs and AD-MSCs differ in potential to differentiate towards UCs. Obtained results suggest that CM induced more changes in selected markers expression in the case of BECs. Unfortunately, CM was not sufficient source of signals to generate UCs-like phenotype. It is an essential but still future goal.

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Keywords: In vitro microenvironments, Multipotent (mesenchymal) stem cells

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Characterization of an injured enthesis: insights on the role of miRNAs in tendon fibrosis and ectopic ossification

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INTRODUCTION: The entheses connecting tendons to bones are frequent sites of injury, and unfortunately, injured entheses typically undergo fibrotic processes that yield scar tissue prone to recurrent ruptures. To tackle the challenge that represents the regeneration of the enthesis upon injury, we characterized the early healing of an injured patellar enthesis in a rodent model using histology, microRNA expression, and in situ hybridization techniques. This allowed us to gain key insights into the processes involved in the improper healing of injured enthesis, which would ultimately lead to the development of novel strategies to improve enthesis regeneration and healing.

METHODS: A longitudinal defect was created at the patellar enthesis of adult rats. Explants were collected at one (n=6) and 10 days (n=6) after the injury. Tissue samples of the contralateral side were used as control. Samples of the injured enthesis were stained with hematoxylin & eosin, safranin O, and alcian blue. Additionally, immunostaining for collagen type I and collagen type II was performed. A miScript qPCR array specific for fibrosis was used to investigate the expression of microRNAs upon injury and in situ hybridization (ISH) was performed to localize the tissue-specific expression of the most de-regulated microRNAs

RESULTS: The histological analysis of the samples harvested 10 days after the injury demonstrated a deposition of a fibrotic-like tissue in the tendon side of the enthesis, characterized by the abundant presence of proteoglycans and disorganization of the collagen fibers of the tendon. We also observed signs of ectopic ossification in the tendon portion of the injured enthesis. This was confirmed by the immunostaining, which highlighted an area of the extracellular matrix of the tendon with a dense deposition of collagen type I surrounded by collagen type II. The miR-16-5p and the miR-133a-3p were the most de-regulated miRNAs upon injury. ISH experiments revealed that the expression of both of these miRNAs was localized in the newly-formed fibrotic tissue and around the ectopic ossification on the samples from timepoint 10 days.

DISCUSSION & CONCLUSIONS: We demonstrated that the normal healing of the enthesis undergoes fibrosis and ectopic bone formation that yields a tissue morphologically different from the native enthesis. Moreover, we have identified two microRNAs with a potential role in the improper healing of the enthesis. Since miRNA's expression can be modulated by using mimics or antagomirs, our results indicate that both miR-16-5p and miR-133a-3p, could be used as potential therapeutic targets

to avoid fibrosis and promote enthesis regeneration.

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Interfaces - biological

PP-158

Designing an immunomodulatory, biomimetic ACL graft based on cellulose nanostructures

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INTRODUCTION: The gold-standard treatment for Anterior Cruciate Ligament (ACL) rupture is an autologous tendon graft, despite a nine-month rehabilitation process with disappointing long-term results[1]. Alternative synthetic polymer grafts have been limited by chronic inflammation[2]. Bacterial cellulose is a crystalline polysaccharide produced by bacteria, exhibiting a hierarchical structure similar to that of ligament tissue. Chemical and mechanical treatments can liberate the individual nanofibrils, referred to as bacterial cellulose nanofibrils (BCNF). Bacterial cellulose nanofibrils (BCNF) have impressive mechanical properties with similar dimensions to collagen fibrils, and can be functionalised with peptides to modulate specific cellular responses. For example, DGEA has been shown to drive macrophages towards an M2 phenotype[3]. This work aims to produce an all-cellulose composite fiber as a synthetic ligament graft material with structural components based on BCNF and carboxymethyl cellulose (CMC). We isolate BCNF and wet-spin BCNF-CMC solutions to produce hierarchical, biomimetic nanocomposite fibres. We then functionalise the surface of BCNF with specific peptides as a method of modulating cellular response.

METHODS: BC was functionalised with carboxylate groups via TEMPO-mediated oxidation, followed by mechanical fibrillation to isolate BCNF. BCNF-CMC solutions were wet-spun using a coagulation bath with aluminium sulphate ($\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$). SAXS, SEM, and tensile testing methods were used to analyse the nanofibrillar alignment, crystallinity, morphology, and mechanical properties of the fibres. RGD and DGEA were conjugated to BCNF via a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) cross-linking mechanism, and cell response assessed via macrophage and fibroblast morphology.

RESULTS: TEMPO-mediated oxidation of BC is shown to be necessary for successful isolation of BCNF, with an average diameter of 34.60 ± 9.43 nm. Rheological measurements showed BCNF to significantly increase the viscosity and shear thinning properties of the CMC-BCNF spinning dope. Nanocomposite fibres were successfully coagulated within the $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ bath with promising alignment of the nanofibrils along the fibre axis.

DISCUSSION & CONCLUSIONS: Fibrillation of TEMPO-BCNF occurs as a result of the mutualistic repulsion of carboxylate groups. The large increase in viscosity of BCNF-CMC spinning dopes is indicative of good interfacial adhesion between the two. Overall, mimicking the hierarchical structure of the ACL appears to be a promising approach to replicating its mechanical and biological performance.

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Keywords: Immunity / immunomodulation / macrophage, Nanomaterials (inc graphene)

PP-159

Development of an in vitro musculoskeletal model using poly (glycerol sebacate methacrylate) (PGSM) polyHIPE-based 3D microporous scaffold encompassing regionally controlled mechanical properties

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INTRODUCTION: Globally, musculoskeletal (MSK) conditions are a leading contributor to disability causing a compromised quality of life. Current treatments provide only temporary relief and pose unwanted side effects. Improvements in treatment are limited by the paucity of relevant experimental models. Animal models are commonly employed in the drug discovery pipeline, however, differences between humans and animals in size, anatomy, and biomechanics limit their clinical relevance. Tissue engineering offers the potential to provide clinically relevant information via 3D study models. This study focuses on the fabrication of a ternary 3D biomaterial scaffold to develop an in vitro musculoskeletal model comprising bone, cartilage, and skeletal muscle tissues.

METHODS: 50% and 80% PGSM polyHIPEs (polymers constituting high internal phase emulsions) were synthesized via the emulsion templating technique [1]. The three regions of the scaffold include 50% PGSM polyHIPE (PGSM-50) for skeletal muscle, 80% PGSM polyHIPE (PGSM-80) for cartilage, and 80% PGSM polyHIPE with 5 wt% hydroxyapatite (PGSM-80-HA) for bone. Scanning electron microscopy (SEM) was used to visualize the microporous structure of the scaffolds and energy dispersive x-ray (EDX) spectroscopy was used to determine the presence and distribution of HA particles in PGSM-80 HA. C2C12 skeletal muscle cells were cultured on PGSM-50 for 7 days. Cell metabolic activity was determined using the resazurin assay and confocal microscopy was used to image cells on scaffolds via live dead staining. Parallel experiments with bone cells on PGSM-80-HA scaffolds are in progress.

RESULTS: SEM micrographs indicate collapsed pores in PGSM-50 while PGSM-80 and PGSM-80-HA retained structural integrity and showed high internal connectivity. The pore surfaces of PGSM-80-HA appear granulated due to HA particles. EDX elemental mapping revealed calcium and phosphorus peaks, indicative of HA. C2C12 cells on PGSM-50 showed comparable metabolic activity with cells on tissue culture plastic. The metabolic activity on day 4 was approximately 4-fold higher than on day 1. Confocal images of the live-dead stained scaffolds showed live cells proliferating within the microporous structure. Additional, ongoing studies characterizing the cellular response to these scaffolds will also be reported.

DISCUSSION & CONCLUSIONS: The results from the resazurin assay and live dead staining suggest that PGSM-50 scaffolds demonstrate good biocompatibility and promote C2C12 cell attachment, proliferation, and migration. Future studies will involve inducing differentiation of cells cultured on these scaffolds to analyse the extracellular matrix formation and assay biomarkers indicative of tissue formation.

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Keywords: Biomaterials, In vitro microenvironments

PP-160

The Bio-Inductive Role of Decellularised Anterior Cruciate Ligament (ACL) Extracellular Matrix in a Polymer-Allogenic Tissue Hybrid Scaffold for Enhanced ACL Reconstruction

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INTRODUCTION: Autologous hamstring and patella-tendon grafts are gold standards for anterior cruciate ligament (ACL) reconstruction [1]. However, reduced strength and flexion, and anterior knee pain limit patient recovery [2]. Bioengineering has utilised non-/biodegradable polymers to promote a regenerative response yielding varied results and commonly revised with a gold standard approach due to graft elongation and poor integration with the surrounding anatomy [2]. Bioengineered electrospun polycaprolactone (PCL)-ligament extracellular matrix (ECM) scaffolds may enhance the biological response omit co-morbidities associated with graft harvest.

METHODS: ACL tissue from juvenile porcine stifle joints was subjected to an initial cleaning process [3,4,5], collated from protocols processing other soft tissue, and cryomilled to form a fine powder. A variety of PCL:ECM solutions were prepared in hexafluoroisopropanol and cast on glass and electrospun into fibre scaffolds. Specimens were characterised to determine initial chemical changes and confirm the presence of ECM. Murine fibroblasts (3T3) were cultured on scaffolds for 10 days to evaluate cellular metabolism, cytotoxicity and adhesion and morphology.

RESULTS: Fourier Transform Infrared Spectroscopy (FTIR) indicated the presence of the Amide I (1624 cm^{-1}) and II (1545 cm^{-1}) bands in ECM [6] as a shoulder to the carbonyl in PCL (1727 cm^{-1}) [7]. Energy Dispersive X-Ray analysis confirmed the nitrogen in PCL:ECM films in addition to sodium and chlorine, mirrored in X-ray fluorescence (XRF). UV-Vis spectra indicated a small increase in absorption of PCL with the addition of ECM. Fibroblast populations increased for all conditions, with stable morphologies after 3 days.

DISCUSSION & CONCLUSIONS: FTIR offers a consistent method for confirming the presence of ECM within PCL scaffolds over other spectra methods. ECM has little surface effect with respect to wettability, likely due to PCL dominating surface chemistry which may currently limit the cellular response. Further characterisation is needed to establish a more robust association between ECM content and cellular viability.

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Keywords: Biomaterials, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-161

Mechanically stimulated 3D in vitro engineered striated muscle constructs

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INTRODUCTION: Muscular dystrophies are a heterogeneous group of diseases that affect the contractile capacity of striated muscle tissue. Some vital human functions such as mobility, locomotion, breathing, and metabolic processes are harmed depending on the severity of each dystrophy. The use of in vitro models is highly relevant to the study of the molecular mechanisms of these pathologies. Typically, monolayer cultures have been widely used. Nevertheless, due to the high structural complexity of the striated muscle tissue, the three-dimensional (3D) models are becoming the gold standard method.

Recently, some strategies and procedures have been described to develop 3D striated muscle constructs, including chemical, electrical and mechanical stimuli. Concretely, mechanical stimulation is very interesting as it is related to the effect of stress during embryogenesis. However, the mechanisms by which these stimuli affect the maturation of the constructs are not fully understood yet.

METHODS: In this context, we present a custom-made culture platform capable of performing different stretch routines on in vitro engineered 3D muscle constructs. This culture setup also provides photo-tracking capabilities to monitor the thickness of the myobundles during development and stimulation. This feature could be used to provide real-time feedback to the control loop and continuously adjust the stretching program applied. C2C12 cells were cultured within a collagen-based hydrogel to generate the 3D-engineered muscle constructs. A differentiation protocol, including specific culture media, was used to promote the formation of myotubes together with mechanical stimulation. Their initial length was set to 20 mm, and a constant uniaxial stretch of 10% and 20% elongation was tested to determine its influence on myotube formation. End-point immunofluorescence imaging was used as a characterization method to evaluate the maturation levels of myotubes within the myobundle. DAPI and Myosin heavy chain (MHC) were used.

RESULTS: A reduction in the 3D constructs diameter was observed during the culture, principally in those stimulated mechanically. This effect relates to the reorganization of the extracellular matrix that myoblasts induced upon fusion. Immunofluorescence analysis revealed MHC expression in all cultures, but myotubes alignment was higher in those stretched.

DISCUSSION & CONCLUSIONS: Even though the morphology and cellular organization are characteristic of muscle, maturity levels still differ from native tissues. Mechanical stimulation does affect myobundle development and opens promising strategies to improve 3D engineering in vitro muscle constructs. Finally, this might lead to more sophisticated native tissue-like models, reduce variability, and facilitate the study of pathophysiological processes related to striated muscle.

Keywords: In vitro microenvironments, Bioreactors

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A 3D in vitro model assembled with stem cells from healthy and tendinopathic human tendons: applications and perspectives

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INTRODUCTION: Poor tendon repair is a serious clinical problem, due to the difficulties in reorganising the complex multiscale hierarchical structure of native tendons (1,2). Tendon stem/progenitor cells (TSPCs) were identified as tendon-specific adult stem cells that exhibit universal characteristics of stem cells and play a key role in tendon homeostasis, repair and regeneration. In this context, TGF- β signaling is essential for stem/progenitor cell recruitment into degenerative tendons. Moreover, TSPCs regulate inflammation during healing of acute tendon injuries. In this study, we proposed a dynamic culture of fibrin 3D scaffold bioengineered with TSPCs to provide an in-vitro model for tenogenesis (3,4). TSPCs were obtained from both healthy and tendinopathic human samples to compare their differentiation potential under perfusion and a proper tenogenic medium.

METHODS: Healthy and tendinopathic tendon samples were obtained from patients undergoing surgery after traumas. human TSPCs (hTSPCs) were extracted during 3 weeks, collected and characterized by flow cytometry (5). hTSPCs were then cultured within 3D scaffolds in a medium supplemented with hTGF- β 1 for 21 days, under dynamic conditions provided by a custom-made bioreactor. Evaluation of cell survival, histological examination, immunofluorescence analysis, as well as quantitative gene expression of different target genes (related to tenogenesis and immunomodulation) were performed. Cytokine levels in the supernatant were also measured by immunoassay.

RESULTS: The results revealed that the 3D matrix exerted a rejuvenating effect on pathological hTSPCs, in a comparable manner to healthy hTSPCs, supporting simultaneously cell survival and proliferation. Moreover, TGF- β signaling, coupled to a dynamic environment, promoted hTSPCs differentiation events, with tendon related genes overexpression (COL1A1, SCX-A, DCN, TNC, TNMD), as confirmed by immunofluorescence analysis. Interestingly, pathological hTSPCs showed different morphological features and an impaired balance of pro-inflammatory and anti-inflammatory

cytokines, when compared to healthy hTSPCs.

DISCUSSION & CONCLUSIONS: The 3D scaffold plus dynamic culture seemed an extremely favorable biomimetic environment to collect data on cells differentiation. Furthermore, both tenogenic and cytokines gene expression of hTSPCs were modulated by the presence of TGF- β 1, suggesting a putative effect on resident stem cells during tendon healing events. The study opens perspectives on TSPCs 3D cultures (healthy and pathological) as an in vitro model matrix for the understanding of their behavior during tenogenic events.

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Keywords: Hydrogels and injectable systems, Bioreactors

PP-163

3D bioprinting of a macroscopic construct with piezoelectric nanoparticles for skeletal muscle tissue engineering applications

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INTRODUCTION: Skeletal muscle tissue engineering (SMTE) aims to promote tissue regeneration using cells, promyogenic materials, and biophysical cues. Among SMTE techniques for macroscopic biofabrication in vitro, extrusion-based 3D bioprinting has emerged as the gold standard. In the literature, however, there are no cm-size constructs with advanced myocytic maturation. The combination of piezoelectric barium titanate nanoparticles (BTNPs) with low-intensity pulsed ultrasound (LIPUS) in 3D-printed bioinks has proved its potential to stimulate myogenic differentiation wirelessly[1].

METHODS: A fibrin-based bioink with 1×10^7 C2C12 cells/mL (*fibroink*) and a sacrificial bioink were produced as previously reported[2]. Rheological characterization of bioinks included: rotational (40-0°C, $\dot{\gamma} = 100\text{s}^{-1}$) or oscillatory (40-0°C, 1Hz, $\dot{\gamma} = 10\%$) temperature sweeps, strain-amplitude sweep (9°C, 1Hz, $\dot{\gamma} = 0.01\text{--}1000\text{s}^{-1}$), and shear rate sweep (9°C, $\dot{\gamma} = 0.01\text{--}1000\text{s}^{-1}$). Bioink storage conditions were tested, followed by rheological tests. For myoblast viability tests, *fibroink* was kept for 2h at 5, 9, 13, or 37°C, crosslinked with thrombin (1U/mg fibrinogen), and myoblast viability was assessed. Printing conditions were optimized by evaluating filament dimensions (parameters assessed: 0.3-0.5-0.7bar; 10-15-20mm/s). BTNPs of 60nm diameter with propylene glycol alginate coating were embedded in *fibroink* at 250µg/mL.

RESULTS: The viscoelastic behavior of sacrificial bioink and *fibroink* was confirmed, and a storage protocol of 24h at 4°C was chosen. For the bioprinting process, the temperature of 9°C was selected based on myoblast viability after 5 days in growth medium. The presence of myoblasts had no effect on the G' , G'' , and viscosity of the *fibroink*. Shear rate sweep tests confirmed the shear-thinning behavior of the sacrificial and *fibroink*. Yield stresses (at $\dot{\gamma} = 0.01\text{s}^{-1}$, start method) resulted in ~6.9Pa (sacrificial) and ~3Pa (*fibroink*). Printing parameters were optimized for suitable nutrient diffusion resulting in 0.5bar, 25mm/s, with fibers of 230×700µm for *fibroink* and 0.5bar, 20mm/s, with fibers of 200×700µm for sacrificial). The BNP-doped *fibroink* did not show rheological differences compared to the *fibroink*. BNP-doped *fibroink* and sacrificial were coprinted making a macroscopic construct (20×10×2.5 mm) on a custom-made platform for LIPUS stimulation.

DISCUSSION & CONCLUSIONS: We developed a reproducible protocol for the preparation of bioinks. We defined the optimal printing parameters with a systematic analysis of myoblast viability at different temperatures and filament dimensions. Finally, we obtained a macroscopic construct with intercalated BNP-doped *fibroink* and sacrificial bioink ready for myocytic differentiation and LIPUS stimulation.

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Keywords: 3D printing and bioprinting, Nanomaterials (inc graphene)

PP-164

A Novel Parathyroid Hormone 1-34 Plasmid Gene Delivery System for Enhanced Bone Repair

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INTRODUCTION: Postmenopausal osteoporosis intensifies risk for impact fractures with complicated healing due to low bone density and tissue fragility. Teriparatide (PTH-1-34) is one of few FDA-approved treatments for osteoporosis; daily subcutaneous treatment of recombinant-PTH-1-34 enhances bone mass and reduces fracture risk(1). The local delivery of recombinant PTH through biomaterials enhanced the osteointegration(2) and healing in critical-sized femoral defects(3). However, previous studies show that recombinant protein biomaterial-delivery (Bone Morphogenic Protein-2) can induce ectopic bone formation and cytotoxicity. This study investigated a new approach, utilizing osteoconductive collagen-nanohydroxyapatite scaffolds(4) to deliver a novel PTH-1-34 plasmid via nanohydroxyapatite nanoparticles to induce endogenous cells to produce PTH-1-34 protein in a sustained and controlled fashion, to upregulate the osteogenic repair responses and heal complicated fractures.

METHODS: PTH-1-34 plasmid and osteogenic nano-hydroxyapatite nanoparticles (NPs), previously optimised for bone applications(4), were complexed and characterized (size, polydispersity, zeta potential by dynamic light scattering). Bone-marrow-derived mesenchymal-stem-cells (MSCs) from healthy and osteoporotic Sprague-Dawley's were maintained in growth (GM) or osteogenic media (OM). Monolayer: Cells were seeded (24h) before transfection and evaluation (PCR at Day-3, DNA, alkaline phosphatase (ALP), calcium at Day-14). Scaffolds: NPs were soak-loaded onto freeze-dried, crosslinked collagen-nanohydroxyapatite scaffolds (4); MSCs were added dropwise to scaffolds and cultured before evaluation of osteogenic capacity (DNA, calcium, Alizarin-Red stain at Day-28).

RESULTS: Nano-sized complexes were formed at 430 ± 182 nm, with low polydispersity and a negative charge (-16.43 ± 1.72 mV). In monolayer, MSCs were transfected effectively with a 50-fold to 10-fold increase in expression with nano-hydroxyapatite and lipofectamineTM, respectively. PTH-1-34 NPs enhanced osteogenesis in MSCs in monolayer, with a 3-fold increase in calcium production (GM and OM) and a 6-fold increase in ALP (GM). This effect was observed in scaffolds resulting in increased calcium and staining-intensity. The impact of PTH-1-34 plasmid on MSCs from osteoporotic animals was equivalent to controls, increasing proliferation. The regenerative potential of scaffolds delivering PTH-1-34 plasmid is currently assessed in a load-bearing rat femoral-defect model.

DISCUSSION & CONCLUSIONS: The study developed an innovative biomaterial-based delivery-system for PTH-1-34 tailored for healthy and osteoporotic bone fractures. The future use of genetic cargoes may significantly improve the quality of healed bone in patients with osteoporosis who report complications and poor outcomes. The scaffold-based system has the potential to become a new 'off-the-shelf' product for healing bone-fractures for osteoporotic subjects.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-165

In Vitro and In Vivo Characterization of Composite Electrospun Scaffolds with ICOS-Fc for Osteoporotic Fractures

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INTRODUCTION: Osteoporosis is a bone disease caused by an imbalance between bone resorption by osteoclasts and bone formation by osteoblasts that makes the bone brittle and increases the risk of fractures. In this context, ICOS-Fc is a recombinant molecule able to trigger ICOS-Ligand (also known as B7h) in osteoclasts and inhibit their bone resorption function stopping the osteoporotic process in bone fracture healing.

Here, we fabricated electrospun composite scaffolds by incorporating nanohydroxyapatite (nHA_Sr) or mesoporous bioactive glasses (MBG_Sr) with strontium ions to boost bone regeneration. Then, ICOS-Fc was grafted on MBG_Sr surfaces or directly on the electrospun fibers. The resulting scaffolds were tested in vitro and in vivo to prove the efficiency of ICOS-Fc to act against osteoporosis.

METHODS: PEOT/PBT composite scaffolds with either ICOS-Fc functionalized MBG_Sr or nHA_Sr were fabricated by electrospinning. MBG_Sr were functionalized by grafting ICOS-Fc (MBG_Sr-ICOS-Fc) to their surface while scaffolds containing nHA_Sr underwent aminolysis and EDC/NHS functionalization to graft ICOS-Fc on the fibers surface (nHA_Sr-ICOS-Fc). An ELISA-like assay was used to assess the functionality and amount of ICOS-Fc grafted on nHA_Sr and MBG_Sr-ICOS-Fc scaffolds.

Human osteosarcoma cell lines U2OS (ICOSL+) or HOS (ICOSL-) were seeded on the electrospun scaffolds and used to study migration in a Boyden chamber. In vivo experiments were performed in a cranial defect model of healthy or ovariectomized (OVX) mice and scaffolds were applied on the open lesion. After 4 weeks, PCR, μ CT for bone volume fraction (BV/TV), mechanical testing and histology were performed.

RESULTS: MBG_Sr-ICOS-Fc and nHA_Sr were incorporated up to 12,5 wt.vol% and 15 wt./vol%, respectively. ICOS-Fc functionality on MBG_Sr-ICOS-Fc was preserved during scaffold fabrication while 15-nHA_Sr-ICOS-Fc scaffolds were successfully grafted with up to 25 μ g/ml ICOS-Fc.

A migration assay using U2OS cells showed that cell migration was inhibited by around 30% and 45% for nHA_Sr-ICOS-Fc and MBG-ICOS-Fc, respectively. By contrast, ICOSL- HOS cells showed no inhibition in the migration.

BV/TV and μ CT results showed new mineralization on the scaffolds surface. In OVX mice, bone

formation in composites scaffolds was higher than control (empty defect). In addition, composite scaffolds decreased the expression of NFAT-c1 osteoclast marker in OVX mice.

DISCUSSION & CONCLUSIONS: In conclusion, ICOS-Fc was successfully incorporated into composite PEOT/PBT-based scaffolds.

In vitro and in vivo results showed how scaffolds with ICOS-Fc can have a dual action by targeting osteoporosis alongside boosting bone regeneration.

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Keywords: Bone and bone disorders (osteoporosis etc), Composite materials

PP-166

Exploring microgel technology for high-throughput formation of osteoclasts

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INTRODUCTION: Osteoclasts are multinuclear cells of hematopoietic origin responsible for bone resorption (1). Due to their crucial role in bone homeostasis and pathologies, osteoclasts are investigated in multiple research areas relating to bone health and disease (2). However, in vitro research on osteoclasts remains challenging due to the intrinsic heterogeneity of the cultures (3). Consequently, there is a need for establishing pure osteoclast cultures. Here, we investigate whether encapsulation of osteoclast precursors in microfluidically generated hollow microgels composed of a tyramine-conjugated dextran shell (4) could facilitate cell aggregate formation and subsequent cell fusion thus forming a single osteoclast with predetermined number of nuclei per microgel. Due to the sacrificial nature of the microgel, generated osteoclasts can be released to obtain pure osteoclast cultures.

METHODS: Primary human mononuclear cells were isolated from buffy coats and differentiated toward macrophages. Macrophages were detached and encapsulated in microgels as described previously (4). Encapsulated macrophages were differentiated toward osteoclasts for up to 29 days. During the culture period, morphology, and viability were observed using light, and fluorescence microscopy. To assess osteoclast formation with confocal microscopy, microencapsulated cells were stained for $\beta 3$ integrin as an osteoclast marker.

RESULTS: We successfully encapsulated primary human macrophages in $\sim 80 \mu\text{m}$ diameter microgels. Cells survived the encapsulation procedure, as was demonstrated by viability of more than 80 %. Although limited aggregation of macrophages occurred, immunofluorescent staining revealed formation of a number of high quality osteoclasts within the microgels.

DISCUSSION & CONCLUSIONS: We successfully managed to differentiate encapsulated primary human macrophages toward osteoclasts. In further studies, we will investigate protocol optimization to augment cellular aggregation rates and increase production throughput to realize scalable production of high quality osteoclasts.

ACKNOWLEDGEMENTS: This study received financial support from the Twente University RadBoudumc Opportunities (TURBO) program.

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2020;6:100047.

Keywords: Bone and bone disorders (osteoporosis etc),

PP-167

Injectable collagen/calcium phosphate microparticles to reconstruct hard tissues

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INTRODUCTION: The bone extracellular matrix comprises a dense scaffold of type I collagen fibrils mineralized with apatite nanocrystals, and is continuously remodeled. However, in cases of large defects or diseases such as osteoporosis and metastatic cancers, the self-healing process of bone is compromised. The current clinical products face notoriously high failure rates mostly because they lack the structure and composition of bone tissue and are not able to fill fractures with complex geometries. We present here a collagen/calcium phosphate hybrid hydrogel, which self-assembles under a physiological environment into a matrix that reproduces the main structural features of native bone.

METHODS: The key steps to form the bone-like matrix are: (i) Synthesis of collagen/calcium phosphate hybrid particles (Col/CaP): an acidic solution of collagen containing Ca^{2+} , PO_4^{3-} and CO_3^{2-} is spray-dried at room temperature. (ii) Gel preparation: The Col/CaP particles are mixed with water forming a gel, which is then injected into a mold. (iii) Formation of bone-like matrix: the mold containing the gel is placed in a bone-like environment formed by a physiological solution reproducing human plasma in terms of ionic and protein composition, at 37.4 degrees for 5 days. The composition and the structure of the scaffold were characterized from the nano up to the macroscale using SEM, TEM, EDX, FTIR and solid state ^{31}P NMR.

RESULTS: The scaffold displays the hallmarks of hard tissues: a dense network of parallel-aligned collagen fibrils reaching long-range ordering (micrometers) and homogeneously mineralized with nanoparticles of carbonated apatite having the same size and morphology of biological apatite. Ultimately, by playing with the composition of hybrid microparticles this simple approach allows targeting the structure and composition of different mineralized tissues such as bone and dentin.

DISCUSSION & CONCLUSIONS: This investigation opens perspectives in biomimetic tissue regeneration and minimally invasive surgeries. After testing the biocompatibility in vivo, the scaffolds capacity in inducing osteogenesis will be evaluated.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-168

Treatment of cytokine-treated osteochondral plugs using Glucocorticoid and Hyaluronic acid in an ex vivo culture model

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INTRODUCTION: The intra-articular injection of glucocorticoids effectively reduces pain and inflammation in knee osteoarthritis (OA) patients. Also, hyaluronic acid (HA) is used to restore lubrication within the joint, acting as a chondroprotective agent. The combination of HA and GC showed benefits in clinical studies, but data on the cellular level and tissue are lacking. Therefore, the study aimed to investigate the chondroprotective and anti-inflammatory effect of GC and HA alone and in combination on cytokine-treated osteochondral (OC) plugs.

METHODS: Harvested OC plugs from bovine knee joints were incubated for one week to equilibrate samples in the ex vivo environment. After one week, treatment with IL-1 β and IL-17 was performed for three days before 10% of the test substances (GC, HA, and GC/HA) were added to the culture medium. After 14 days, cells within the tissue were analyzed concerning their metabolic activity and expression of anabolic and catabolic genes. Also, thin cartilage sections (100 μ m) were prepared for Live/Dead staining. Histology for each condition was performed, while the supernatant was used to determine sulfated glycosaminoglycans (sGAG) and released pro-inflammatory cytokines. The coefficient of friction was determined by performing tribological tests using a cartilage-on-cartilage test setup.

RESULTS: Anabolic genes were increased by treating OC plugs with GC or the GC/HA combination. Also, the two substances lead to a substantial reduction of catabolic gene expression. Metabolic activity of the chondrocytes was elevated in the GC group. Treatment with cytokines alone leads to a loss in proteoglycan content of the cartilage tissue. This was further reduced by adding GC or GC+HA to the culture medium. Live/Dead staining showed dead cells in the surface layer of the cartilage, which was treated with cytokines alone. sGAG content in the culture medium was highest in the cytokine group without test substances, possibly due to cartilage degradation. Cytokine treatment alone leads to a higher COF than the control group, while adding HA to the inflammatory condition reduced this effect.

DISCUSSION & CONCLUSIONS: Supplementing cytokine-treated OC plugs with a combination of glucocorticoid and hyaluronic acid showed the best results, as cartilage-specific gene expression was increased and genes for degradative enzymes were decreased. Also, cells survived, and tissue was protected from proteoglycan loss and surface degradation. Only COF was higher than in comparison with cytokine treatment alone or adding HA.

ACKNOWLEDGEMENTS: The work was funded by Anika Therapeutics Inc. and has been carried out within the University for Continuing Education.

Keywords: Cartilage / joint and arthritic conditions, Advanced therapy medicinal products

PP-169

Osteochondral regeneration using multi-layered cryogel and chondrocyte-derived extracellular vesicles in a goat model

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INTRODUCTION: Cartilage tissue and underlying subchondral bone get damaged at load-bearing regions like knee and hip due to unbalanced loading, trauma, and disorders such as rheumatoid arthritis and osteoarthritis. Once the degeneration gets started, regeneration becomes very challenging since cartilage is an aneural, avascular, alymphatic tissue and chondrocytes embedded in lacunae fail to migrate to the defect site. Although, in case of osteochondral injury Mesenchymal Stem Cells (MSCs) from bone marrow become available, however the tissue formed is fibrocartilage. So, to drive the chondrogenesis and osteogenesis in cartilage and bone part, a biocompatible, mechanically stable, scaffold is required which can provide suitable niche to the MSCs to differentiate and proliferate. Further, extracellular vesicles derived from chondrocytes aid in chondrogenesis via paracrine signalling.

METHODS: The multi-layered cryogel was fabricated using aligned cooling apparatus to make top, deep, and interface layers of the cartilage and random cooling to make the middle cartilage layer and subchondral bone layer. Cartilage layers were made using chitosan, gelatin, and chondroitin sulfate, while the interface and subchondral bone were composed of nano-hydroxyapatite and gelatin. Further, extracellular vesicles especially exosomes were isolated from the chondrocytes using ultrafiltration method. The scaffold and exosomes were characterized and implanted in medial femoral condyle of the goat knee where a 10 mm deep and 8 mm wide critical size osteochondral defect was created. The control was left without any treatment for the entire period. The regeneration was evaluated using ICRS scoring, Magnetic resonance Imaging (MRI), X-Ray radiography, and micro-CT. Histological staining by hematoxylin and eosin (H&E) and Masson's trichrome (MT) were performed. Gene expression and immunohistochemical studies were done to assess the functionality of the regenerated tissue.

RESULTS: The macroscopic and MRI results showed formation of hyaline like cartilage at the defect site while X-Ray and micro-CT showed enhanced subchondral bone formation. H&E staining showed better cell infiltration and MT showed enhanced collagen deposition in the treatment group. Gene expression studies showed upregulation of aggrecan, collagen II, SOX9 genes in cartilage and collagen I, RUNX2 upregulation in subchondral bone. Immunohistochemical staining also corroborates the same.

DISCUSSION & CONCLUSIONS: The results showed the potential of exosome-laden scaffold to aid in osteochondral regeneration. This combinatorial technique can be commercialized and has the potential to be utilized for treating severe cartilage injuries.

ACKNOWLEDGEMENTS: Department of Biotechnology: Ph.D fellowship

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Keywords: Biomaterials, In vivo and animal models

PP-170

In situ regeneration of nasal septal defects using acellular cartilage enhanced with platelet-derived growth factor

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INTRODUCTION: The need for reconstructive surgery of nasal septal cartilage (NSC) commonly occurs due to the obstruction of nasal breathing, trauma, and neoplasms. The damage of the NSC may lead to septal perforation associated with a disturbance of nasal breathing and chronic nasal discharge. Autologous tissues, such as cartilage in combination with chondromucosal bridge flaps are mainly used for the closure of septal defects. However, the repair is still challenging and not always successful.

METHODS: Here we used porcine decellularized NSC (DNSC) for the reconstruction of the nasal septum in rabbits. DNSC was functionalized with recombinant platelet-derived growth factor-BB (PDGF-BB). The uptake and release of PDGF-BB were measured by ELISA and confirmed by in vitro migration experiments. The in vivo study consisted of three experimental groups, receiving 1) PDGF-BB-laden or 2) untreated DNSC, and 3) autologous transplant (control) respectively. The animals were sacrificed after 4 and 16 weeks. Septal deviation, thickness, and shrinkage of septal transplants were analyzed by Magnetic Resonance Imaging (MRI). Histological analysis was used for the evaluation of transplant biocompatibility and assessment of the NSC regeneration.

RESULTS: An uptake of PDGF-BB by the DNSC was followed by its gradual ongoing release as measured by ELISA. Migration studies confirmed that the chemotactic potential of PDGF-BB following the DNSC binding was retained.

The MRI revealed no significant transplant shrinkage or septal perforation at 4 or 16 weeks and only minor septal deviations irrespective of the study group. Histological analysis of biocompatibility showed slight irritation caused by the DNSC transplants, as compared to the autologous controls. The integrity of the DNSC transplants was preserved after 4 weeks, showing only limited regions of degradation, often accompanied by de novo cartilage formation in the vicinity. After 16 weeks, a large portion of DNSC was degraded, and increased neocartilage formation was observed.

Degradation of DNSC was more pronounced in untreated as opposed to the PDGF-BB-laden matrices. Conversely, significantly more neocartilage formation was observed in the PDGF-BB group. No degradation or neocartilage formation were observed in autologous control at any time point.

DISCUSSION & CONCLUSIONS: DNSC effectively maintained the supportive function of the septum. Good biocompatibility of transplants was observed. PDGF-BB functionalization enhanced de novo cartilage formation and delayed the degradation of DNSC scaffolds.

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Keywords: Decellularised matrices, In vivo and animal models

PP-171

Biotechnological unsulfated chondroitin features, alone and in combination with hyaluronic acid and PRP for tissue regeneration: in vitro model based on pathological joint derived human cells and stem cells

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INTRODUCTION: Osteoarthritis (OA) is characterized by pain and articular cartilage progressive degradation. A diffuse treatment is viscosupplementation, often based on glycosaminoglycans injections such as hyaluronic acid (HA) and chondroitin sulfate (CS). Recently, exploiting a biotechnological process, a pharma grade unsulfated chondroitin (BC) was obtained [1]. BC proved beneficial biological effects in comparison to CS, in different OA in vitro models [2,3].

Here, BC was combined to HA and eventually to PRP to assess the improvement in rheological features and biological effects in OA articular cells. In addition, BC was chemically modified, even in presence of HA, to improve the mechanical performance and to slower enzymatic degradation.

METHODS: Platelet rich plasma (PRP) was characterized at molecular level and lyophilized. The hydrodynamic characterization was performed by size exclusion chromatography coupled to triple detector (SEC-TDA- laser scattering, Intrinsic viscosity and refractive index). XBC and XHA/XBC were prepared by a crosslinking reaction [4] and the chemical modification was assessed by Nuclear magnetic resonance (NMR). Mechanical features were evaluated by a rotational rheometer (Anton Paar Physica MCR 301). Stability was studied in the presence of Bovine testicular hyaluronidase (BTH). Finally, human primary cell cultures were assessed and an extensive molecular fingerprint obtained (RT-PCR, Western blot-WB, Immunofluorescence-IF) to comparatively evaluate treatments.

RESULTS: As expected, the linear combinations and the modified/crosslinked ones showed different mechanical performances and the latter proved high resistance to the BTH attack and the chemical modification did not affect biocompatibility. The combination of HA/BC with PRP revealed a consistent rheological profile, useful for the application in intra-articular injections. In addition, the expression of specific chondrocytes biomarker (collagen II) was prompted by the formulation developed as showed by qRT-PCR, WB and IF. Interestingly, all gels were effective in the reduction of pro-inflammatory cytokines (IL-6, NF- κ B), matrix degradation related biomarkers, (metalloproteases MMP-13, TIMP-1).

DISCUSSION & CONCLUSIONS: The outcomes showed that the combination of BC with HA and PRP improves the viscoelastic behavior and has anti-inflammatory effects. Biofermentative GAGs crosslinking results in better mechanical features and stability, whilst preserving biocompatibility.. BC can be a novel bioactive polymer for regenerative purposes in OA and cartilage repair.

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Keywords: Biomaterials, Hydrogels and injectable systems

PP-172

Prostaglandin E2 inhibits hypertrophy during mesenchymal stromal cell chondrogenesis

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INTRODUCTION: Mesenchymal stromal cells (MSCs) are a promising chondrocyte source for cell-based cartilage repair strategies; however, MSC chondrogenesis yields a hypertrophic rather than an articular chondrocyte phenotype in vitro. Drivers of this mis-differentiation remain insufficiently identified so that attempts to generate sustainable, phenotypically stable MSC-based neocartilage fail and impede clinical translation. In the search for drivers of hypertrophy, inhibition of the prostaglandin E2 (PGE2) synthesizing enzyme cyclooxygenase-2 (COX-2) during ATDC5 chondrogenic differentiation was found to decrease the expression of BMP-2-induced hypertrophic markers. Intriguingly, it was recently shown that PGE2 secretion is higher during MSC in vitro chondrogenesis compared to articular chondrocytes, suggesting PGE2 as a putative regulator of chondrocyte hypertrophy. The aim was to investigate the cause of high PGE2 release by differentiating MSCs and determine whether modulation of PGE2 levels affects chondrocyte hypertrophy, thereby testing the idea that PGE2 could be a promising target for generating phenotypically stable MSC-based neocartilage.

METHODS: Human bone marrow-derived MSCs were cultured as 3D pellets under TGF β stimulation (10ng/mL) and treated with PGE2 (1 μ M), the COX2 inhibitor celecoxib (0.5 μ M), the PGE2 receptor antagonists AH23848 or AH6809 (10 μ M), or DMSO as control (n=3 and 2 for AH23848, respectively). MSC chondrogenesis and hypertrophy were assessed at day 42 of differentiation using proteoglycan deposition (histology), expression of cartilage and hypertrophy markers (qPCR), and ALP activity assay. PGE2 secretion upon TGF β withdrawal (from day 21 on, n=2) was measured via ELISA.

RESULTS: TGF β deprivation led to a striking decrease in PGE2 secretion, identifying TGF β as a PGE2 driver. Homogenous deposition of proteoglycans and strong increases in COL2A1 expression revealed that MSC chondrogenesis was not affected by PGE2 level modulation. Importantly, while PGE2 significantly reduced the expression of hypertrophy markers (COL10A1, ALPL, IBSP, and SPP1) compared with the DMSO control, the inhibitors oppositely raised their levels, albeit partially only by trend. Additionally, PGE2 significantly decreased ALP activity in the supernatant (3.2-fold), whereas celecoxib and AH6809 caused a slight but significant and AH23848 a tendential increase (1.1-fold, 2.0-fold, 2.0-fold, respectively). This identified PGE2 as an important inhibitor of chondrocyte hypertrophy.

DISCUSSION & CONCLUSIONS: Our data identified for the first time PGE2 as a factor that can stabilize the chondrocyte phenotype and is putatively part of the antihypertrophic TGF β activity during MSC chondrogenesis. This revealed PGE2 as an attractive candidate for new approaches to suppress chondrocyte hypertrophy, e.g. in combination with known antihypertrophic agents like PTHrP pulses or WNT inhibition in order to enhance MSC-based cartilage engineering.

Keywords: Cartilage / joint and arthritic conditions, Multipotent (mesenchymal) stem cells

PP-173

Rapid Evaporative Ionization Mass Spectrometry (REIMS) with diathermic knife as a potential diagnostic tool in cartilage defect and osteoarthritis patients

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INTRODUCTION: Young adults following knee injury (e.g. cartilage defects) have a severely increased risk for development of osteoarthritis later in life if not treated accordingly. Adequate treatment of these defects and phenotyping of these patients can contribute to early detection of osteoarthritis and is critical for a delay in osteoarthritis development. Rapid Evaporative Ionization Mass Spectrometry (REIMS) coupled to a diathermic knife has been used as a method for drug detection, tissue identification and classification, and could function as possible prognostic or diagnostic tool. The lipid metabolism has already been shown to play an important role in osteoarthritis pathology and might therefore act as an important class of molecules for biomarker discovery. In this study, we analyzed the lipid profiles in Hoffa's fat pad (HFP) of cartilage defect and osteoarthritis patients using REIMS for analysis of electrosurgical vapors. We aimed to identify specific lipid profiles that discriminate between cartilage defect and osteoarthritis patients.

METHODS: 15 snap-frozen HFP explants, collected during cartilage defect surgery or total knee replacement, were thawed on ice and measured at 5 different locations with a diathermic knife linked to a REIMS ionization source coupled to a XEVO G2-XS qTOF to collect lipid spectral profiles. Lipid profiles of HFP in negative ion mode were obtained for all samples and subsequently the lipid data of the 75 measurement points was analyzed using principal component analysis/linear discriminant analysis (LDA). Cross validation with confusion matrix was performed to test the model for correct classification.

RESULTS: The LDA showed clear separation of the patient groups, despite the tissue heterogeneity of HFP. In addition, specific lipids contributed to the discrimination between osteoarthritis and cartilage defect patients.

DISCUSSION & CONCLUSIONS: To our knowledge, this is the first study using REIMS on HFP as

possible diagnostic and prognostic technology in this research field. With the information we have gathered, we plan to expand our measurement groups to improve the classification model and correlate this information with MRI, questionnaires and other relevant patient characteristics (e.g. BMI). Further development of this technique and its use in the orthopedic operating theater could contribute to better understanding of patients regenerative potential after a cartilage defect, better surgical outcome, and delay in osteoarthritis development.

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Keywords: Cartilage / joint and arthritic conditions, Knee

PP-174

Direction Dependent Mass Transport Through the Cartilage Endplate

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INTRODUCTION: Intervertebral Disc (IVD) Degeneration can result from chemical changes in the Cartilage Endplate (CEP) and it might suggest that pain is related to CEP weaknesses and imperfections. The CEP has a crucial role in keeping the IVD healthy by acting as the main gateway of nutrients and waste in and out of that avascular region. Yet, among the spinal tissues, CEPs receive the least amount of attention in scientific literature.

The purpose of this study is to follow a combined in vitro and imaging-driven in silico approach to obtain an improved understanding of the CEP functionality regarding mass transport.

METHODS: In vitro: 6 CEPs were harvested from a fresh bovine tail. Eight mm diameter biopsy samples were prepared and enabled to free swell prior to testing in Dulbecco's Modified Eagle Medium (DMEM). Each sample was fitted tightly into a silicone tube with constant inlet and outlet pressures. DMEM was passed for 10min twice in the forward and reverse direction corresponding to the flow directions into and out of the IVD in vivo respectively. The amount of passed fluid was collected to determine the flow rate.

In silico: The samples were incubated in 40% Hexabrix, a contrast agent, for 48 hours and then imaged by a GE Nanotom[®] M nanoCT device. From the reconstructed nanoCT images, 1 mm diameter diameter subsamples were selected to create 3D models of the pore structure at different locations in the CEPs. To date, one model was meshed and imported into OpenFOAM[®] to perform Computational Fluid Dynamics (CFD) Simulations. The other models are still under development.

RESULTS: The in vitro experiment showed that the average flow rate through that CEP samples was 6.86 mm³/sec and 4.84 mm³/sec in the forward and reverse directions respectively meaning that the reverse flow was 70.55% of the forward flow. The CFD analysis on one subsample showed that the flow rates were 15.1 mm³/sec and 10.7 mm³/sec meaning that the reverse flow was 70.86% of the forward flow.

DISCUSSION & CONCLUSIONS: The results from both the in vitro experiment and the in silico simulation showed that the CEP has a tendency to resist flow differently according the direction of the flow where flow into the IVD was higher than that out of the IVD. This combination of image-based in silico modelling with in vitro experiments is a first step towards a better quantification of the mass transport across the CEP in and out of IVDs.

Keywords: Cartilage / joint and arthritic conditions, In silico models

PP-175

Macrophages for IVD hernia therapy

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INTRODUCTION: Intervertebral disc (IVD) degeneration and herniation is a leading cause of disability globally and a large unmet clinical need. Macrophages are immune cells which may be key targets for the development of IVD therapies. This study establishes the central role of macrophages in IVD spontaneous hernia regression and provides the first preclinical demonstration of a macrophage-based therapy for IVD herniation.

METHODS: Bone marrow monocyte-derived macrophages were administered intradiscally in vivo into a rat model of IVD herniation. Control group consisted in lesioned animals without macrophage administration. N=6 animals per group. The tissue herniated area was quantified in proteoglycan/collagen histological sections at 2 and 6 weeks post-lesion. The systemic effects were evaluated in blood and spleen cells by flow cytometry analysis and in the plasma by cytokine array and proteomics.

RESULTS: Bone marrow-derived macrophages were successfully administered into rat IVD hernias resulting in a 44% decrease in hernia size. No relevant systemic immune reaction was identified by flow cytometry, cytokine or proteomic analysis. Furthermore, a possible mechanism for macrophage-induced hernia regression and tissue repair was unveiled through IL4, IL17a, IL18, LIX and RANTES increase.

DISCUSSION & CONCLUSIONS: This study represents the first preclinical proof-of-concept of macrophage-based therapy for IVD hernia regression. This immunotherapy relies on the potentiation of the physiological hernia regression mechanisms. Further development of this macrophage-based therapy for clinical application is warranted.

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Keywords: Advanced therapy medicinal products, Cell therapy

PP-176

The potential of pre-conditioned human nasal chondrocytes for nucleus pulposus repair

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INTRODUCTION: The success of cell-based intervertebral disc (IVD) therapies is still limited, also because of degenerative environment that restricts the survival and function of therapeutic cells. Spheroids generated from human nasal chondrocytes (NCS) can survive in microenvironment simulating degenerative disc disease (DDD). However, the function of NCS for therapeutic use should still be enhanced.

METHODS: We have developed two pre-clinical models that closely recapitulate early-stage DDD and used them to optimize the function of NCS.

RESULTS: Our in vitro degenerative 3D NP micro-tissue (μ T) model contains pre-degenerated human NP cells with cell-produced matrix in hypoxia, low glucose, acidity, and low-grade inflammation. In the NP μ T model, NCS performed superior compared to NC suspension but still showed reduced viability. In order to enhance NC/NCS function, anti-inflammatory and anabolic drugs were used for NC/NCS pre-conditioning. Among the different compounds tested, only IL-1Ra pre-conditioning inhibited the expression of inflammatory/catabolic mediators and reduced matrix loss compared to non-preconditioned NCS. Our ex vivo model is based on chondroitinase ABC (ChABC)-digested and mechanically-loaded bovine IVDs. Injecting pre-conditioned NCS into this model, we are able to determine their tissue localization, integration, as well as number of pre-conditioned NCS providing optimum function. Data are currently being analyzed.

DISCUSSION & CONCLUSIONS: In summary, both NP μ T model and ChABC ex vivo model recapitulate early-stage IVD disease. We demonstrated that spheroidal organisation of NC is better suited for early-stage NP repair than NC suspension and that pre-conditioning further improves NCS function. Safety and efficacy of NCS therapy will be evaluated in follow-up animal study.

ACKNOWLEDGEMENTS:

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Keywords: Bioreactors, Cell therapy

PP-177

In-Vitro and In-Silico Investigation of Dynamic Compression on Cartilage Endplate Cells in Agarose

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INTRODUCTION: The cartilage endplate (CEP) covers the top and bottom of the intervertebral disc (IVD) and acts to transmit compressive loads and transport water, nutrients, and waste in and out of the disc. Early cartilage endplate (CEP) degeneration is likely to play a key role in IVD degeneration, but little is known about CEP mechanobiology and its changes in degeneration. Investigating these changes is essential to elucidate how the CEP contributes to IVD pathology.

METHODS: *In-vitro*: Bovine-tail CEP cells were expanded until passage three. Afterwards, a 1:1 mixture of CEP cells and agarose was pipetted into silicon molds to create 2% agarose and 1x10⁷ cells/ml carriers, 6 mm diameter and 3 mm thickness, and cultured two days for phenotype recovery. Cell-agarose carriers were placed in custom-made chambers, stimulated with 10 ng/ml TGF- β 1 throughout the entirety of the experiment and dynamically compressed up to 7% strain for one hour at 1.5 Hz every day for up to 14 days. Those not dynamically loaded experienced the constant weight of the chamber lid exerting ~5.1 Pa per carrier. Carriers were collected on Days 0, 7, and 14 for downstream analysis of cell viability, gene expression, and glycosaminoglycan (GAG) content.

In-silico: A 2D axisymmetric porohyperelastic, compressible, Neo-Hookean finite element model (FEM) of a cell-agarose carrier was developed in Abaqus using literature-derived material properties and loaded with dynamic compression as in the *in-vitro* experiment. A previously developed mechanotransduction network model was used to predict protein activation levels by initial mechanoreceptor perturbations standing for dynamic compression (α 5 β 1, α v β 3), physioosmotic pressure (TRPV4), tensile strain (α v β 5), plus chondrogenic media (TGF- β). Predicted protein activation was normalized by baseline conditions.

RESULTS: After seven and 14 days of culture, cell-agarose carriers in all conditions demonstrated significantly increased expression of anabolic genes aggrecan (*ACAN*) 2-200x, collagen II (*COL II*) 3-1000x, and GAG/DNA content 2.5-5x, alongside decreased expression of catabolic gene matrix metalloproteinase 3 (*MMP3*). Reaction forces from FEM (0.07N) matched force data collected during loading (0.06N). The FEM showed that hydrostatic pressure varies from center to edge of carrier. General trends of increased/decreased gene expression and protein activation matched between experimental and network model results.

DISCUSSION & CONCLUSIONS: A novel framework coupling 3D cell culture with *in-silico* methods is presented, in which the FEM provides details about loads experienced at each point within the carrier, while the network model uses these mechanical cues and environmental perturbations to predict protein expression and identify key proteins for future analysis.

Keywords: Intervertebral disc / spine and their disorders, Biomechanics / biophysical stimuli and mechanotransduction

PP-178

3D in vitro model of bladder cancer for evaluation of fluoroquinolones cytotoxic properties

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INTRODUCTION: 3D cell culture better reflect in vivo conditions. The anticancer properties of fluoroquinolones and high concentration which they achieve in urine, may help in bladder cancer therapy. Comparative studies analyzing the cytotoxic effects of norfloxacin, enrofloxacin, moxifloxacin and ofloxacin on normal and cancer uroepithelial cell lines were performed using 2D and 3D culture methods.

METHODS: Experiments using 2D culture included evaluation of drugs cytotoxic properties using MTT assay for calculation of lethal concentrations causing death of 10%, 50% and 90% (LC10, LC50, LC90) used in further experiments. Real-time cell growth analysis was performed to confirm results obtained by MTT assay. Fluorescence and light microscopy was used for evaluation of changes in morphology and cytoskeleton organization. Flow cytometry, luminescence assay and changes in gene expression was used for apoptosis detection and changes in cell cycle phases. 3D culture was performed using low-attachment plates. Luminescence assays were performed for evaluation of cell viability and caspase 3/7 detection. Confocal microscopy was used for analysis of cytoskeleton organization.

RESULTS: All tested fluoroquinolones decreased viability of both tested cell lines in dose- and time-dependent manner in 2D culture. LC10 concentrations not influenced on cell morphology and cytoskeletal organization, in higher concentrations destruction of the actin cytoskeleton and shrinkage the nucleus were visible. Flow cytometry analysis showed inhibition of cell cycle in G2/M phase in bladder cancer cell line, in the case of normal uroepithelium cells this influence was minimal. In both tested cell lines increase number of late apoptotic cells were observed. Molecular analysis showed changes in BAX, BCL2, TP53, and CDKN1 expression in tested cell lines, the downregulation of topoisomerase II genes (TOP2A and TOP2B) was noticed. In 3D culture significant decrease of viability was observed in LC90 concentration, what was accompanied with increase activation of caspase 3/7. Also in LC90 concentration, shrinkage and degradation of actine cytoskeleton was observed. These effects were more visible in cancer compared to normal uroepithelial cell line.

DISCUSSION & CONCLUSIONS: Cells cultured in 3D conditions were more resistant to tested drugs compared to standard 2D culture. Comparing directly effectiveness of tested drugs the most promising properties had norfloxacin and enrofloxacin. These two fluoroquinolones exhibit the highest cytotoxic properties against both tested cell lines, and in the case of norfloxacin almost all calculated LC values for bladder cancer cell line are achievable in urine.

Keywords: Disease models,

PP-179

Fibrous 3D Scaffold for In Vitro Cancer Modelling

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INTRODUCTION: Cancer is one of the leading causes of death worldwide, resulting in almost 10 million deaths in 2020. In vitro modelling of tumour environments allows an investigation of treatment methods and efficient selection of pharmaceuticals. 3D cell culture scaffolds are up-and-coming modelling platforms alternative to traditional 2D ones, providing a more favourable environment to cell attachment, proliferation, and formation of 3D structures. Such scaffolds offer a high surface-to-volume ratio, high porosity, controlled mechanical properties, and ability to provide scaffold-cell and cell-cell interactions.

METHODS: Fibrous 3D cell culture scaffolds were produced by 3D fibre printer (3Df-01C, Bious Labs, Lithuania), which is a merger of melt electrospinning and fused deposition modelling [1]. A nanofibrous layer was added by a traditional electrospinning at the bottom of the scaffold to prevent cell migration away from scaffold. The hydrophilicity of the scaffolds was increased by processing in low-temperature plasma. The cytotoxicity of the scaffolds was evaluated according to ISO 10993-5:2009 using mouse fibroblast cells L929. The scaffolds were cultivated with human breast cell line MDA-MB-231 and glioblastoma cell line U87-MG.

RESULTS: Three different scaffolds were formed possessing fibres and pores of increasing sizes, namely fibre diameters ranging from 8 µm to 25 µm, and pore diameters from 35 µm to 155 µm. The hydrophilicity was adjusted to water contact angle of 60°. This is supported by FTIR analysis, which did not indicate any breakdown of PCL during several stages of thermal processing. In majority of cases, the viability of human breast cell line MDA-MB-231 and U87-MG was greater or at least equal to that of 2D cultures during the 7 day period, as indicated by MTT test. Moreover, cells were able to attach to the scaffold fibres/walls and yielded a high survivability rate. MDA-MB-231 featured an elongated shape, which is closer to their natural shape, as opposed to spindle shape in 2D environments.

DISCUSSION & CONCLUSIONS: 3D fibre printing appears as a very viable method, resulting in scaffolds with cell-appropriate morphology, low cytotoxicity and superior mechanical properties. A range of morphologies provide opportunities for modelling different tumour environments, where cells remain viable for a prolonged time and proliferate. Such systems will further will be challenged with anti-cancer drugs to explore the applicability to the drug selection process.

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Keywords: Cancer,

PP-180

Dynamic in vitro model of Ovarian Cancer

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INTRODUCTION: Ovarian Cancer (OC) research is limited by the lack of an appropriate in vitro tumor microenvironment model. We utilized a perfusion bioreactor (1), at velocities mimicking the cellular conditions in the extracellular fluid flow (2), to construct a new OC model. Vertical perfusion flow was applied, instead of horizontal flow to allow better homogenous cultivation.

METHODS: Velocities and shear stresses throughout the reactor body were simulated by ANSYS Fluent, at different velocities, of vertical or horizontal flow. OC cells were seeded into alginate macroporous scaffolds and cultured in the perfusion bioreactor for 3 days under the simulated conditions. Cell viability was examined by Presto Blue assay. Cell constructs were stained with Phalloidin and Hoechst, imaged, and cell spheroids' sizes were quantified. RT-PCR analysis was conducted for OC-associated Sphingosine-1-Phosphate receptors (S1PRs) genes, and their mRNA expression levels were compared to other culture methods (monolayer, static seeded scaffolds, spheroids), and to OC samples of primary tumor and effusions.

RESULTS: ANSYS Fluent simulation indicated higher homogeneity at 50mL/h vertical flow, compared to horizontal flow conditions. ES-2 seeded scaffolds culture at 50 mL/h vertical flow, resulted in more homogenous cellular viability, compared to horizontal flow; supporting the simulation. The spheroids in the dynamic model were significantly larger, and their actin stress fiber intensity was significantly lower, compared to the static model ($p < 0.05$). mRNA expression levels of S1PR1 and S1PR2 were shown to be significantly lower when 500 mL/h volumetric velocities were applied, compared to 50 mL/h and static conditions ($p < 0.05$); emphasizing the necessity of specific velocity for the OC in vitro model. OC-seeded scaffolds, cultured at 50 mL/h resulted in S1P receptor mRNA expression levels, similar to those of the primary OC samples, compared to other static models.

DISCUSSION & CONCLUSIONS: Vertical perfusion flow at a relatively low velocity was found to be efficient for constructing novel in vitro models of primary OC.

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Keywords: Cancer, Bioreactors

PP-181

Perfusion bioreactor as a perspective platform for rapid and personalised drug-testing on patient-derived prostate cancer tissue

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INTRODUCTION: Innovative ways to personalise treatment for advanced prostate cancer (PCa) are needed. Standard in vitro models fail to replicate the tumour microenvironment (TME), while animal models are expensive, time consuming and ethically discouraged. We have previously shown that ex vivo perfusion culture of cancer tissue enables the preservation of epithelial and stromal cells within extracellular matrix(1,2). Here, we aim to adopt the model for personalised drug testing in PCa.

METHODS: We cultured PCa specimens with Gleason score (GS) 6-7 (low) to 9 (high) for 7 days under perfusion or in static conditions. Cultured tissue was compared with matched fresh samples (D0). Samples were cultured with 0.1 nM or 1 nM dihydrotestosterone (DHT) and selected samples were treated with Enzalutamide (1µM) for 5 days. Tissue maintenance and effects of drug treatment were assessed via immunohistochemistry and immunofluorescence. The expression of the luminal marker CK8 was used to quantify luminal cell density (LCD) as number of CK8+ luminal cells per mm² of tissue area.

RESULTS: We observed morphological differences in the TME in static and perfused conditions, the latter better resembling the D0 and exhibiting less evidence of apoptosis. After 7 days of perfusion, epithelial cells retained androgen receptor (AR) expression. After 7-days culture in 1nM DHT conditions of low GS samples (n=3), we observed a trend towards a lower LCD in cultured vs. D0 tissues (265 vs 1274 cells/mm², p>0.99). In contrast, in a high GS sample (Fig.1), no difference was found in terms of LCD in cultured vs. D0 (695 vs 808 cells/mm²). Culturing this sample in 0.1 nM DHT led to a reduction in LCD (162 cells/mm²), suggesting that PCa cells may still be dependent to androgens in our system. As a proof-of-concept, we tested the effect of the AR signalling inhibitor Enzalutamide in the GS9 sample. We observed a decrease in LCD in the Enzalutamide-treated sample when comparing 1nM DHT vs D0 (288 vs 808 cells/mm²) and an even higher decrease in LCD was observed in 0.1 nM DHT vs D0 (100 vs 808 cells/mm²).

DISCUSSION & CONCLUSIONS: Collectively, these results suggest that the system enables to maintain different hallmarks of PCa, including AR signalling, and is a promising tool for personalised drug testing in PCa patients. Further studies should improve reproducibility in maintenance of luminal cells.

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Keywords: Cancer, Bioreactors

PP-182

Perfusion-based 3D in vitro model to study the interaction between prostate cancer and endothelial cells in the bone metastatic niche

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INTRODUCTION: Metastatic prostate cancer (PCa) has a 5-year survival rate of 31%¹ with a strong propensity to metastasise in bone, inducing a pathological deposition of mineralised tissue. In bone, the endothelial cells (ECs) contribute to such sclerotic lesions through endothelial-to-mesenchymal transition (EndoMT)². We propose an in-vitro model of a vascularised osteoblastic niche (VON) grafted with PCa cells to recapitulate features and microenvironmental cues of the bone metastatic niche, to shed light on the pathophysiological relation between PCa and EndoMT.

METHODS: VONs were generated by culturing 7.5×10^5 cells from human adipose stromal vascular fraction (SVF) in osteogenic aMEM-based medium supplemented with 2 ng/mL fibroblast-growth-factor-2 (FGF2) for 3 weeks, adding 3.0×10^5 PCa cells (LNCaP cell line) at day 15. Co-culture was done on a commercially available hydroxyapatite (HA) porous scaffold under perfusion flow. A pressure sensor was used to measure matrix deposition during culture. Flow cytometry and confocal immunofluorescence (IF) were used to assess the expression of endothelial (CD31), osteogenic (RunX2, osteocalcin), PCa (PSMA, E-Cad) cells, and proliferation (Ki67) markers.

RESULTS: VONs expressed RunX2 and osteocalcin, showing successful osteogenic differentiation of SVF cells. VONs cultured in osteogenic medium exhibited growing resistance to flow during culture, indicating a higher extracellular matrix deposition. The CD31+ ECs in SVF adhered to the HA scaffold and generated branching microvasculature. A population of PSMA+ cells was identifiable in the VON 24h after co-culture, although only 15-20% seeding efficiency was achieved. However, we could observe deep engraftment of PCa in the VON after one week, with LNCaP cells penetrating the scaffold's surface and forming aggregates. Co-localization of E-Cad+ and CD31+ cells was observed, hinting an interaction between PCa and ECs.

DISCUSSION & CONCLUSIONS: We propose a platform recapitulating features of the PCa-bone metastatic niche. It is a promising model for studying tumor-induced sclerotic osteogenesis via EndoMT. Based on our results, we are developing a scaffold designed with porosity varying radially from 90% to 70%, to improve seeding efficiency while incrementing trabecular bone biomimicry³. Next step, we will employ our new scaffold to improve PCa-EC interaction, and microenvironment-driven tumor niche heterogeneity.

ACKNOWLEDGEMENTS: Project funded by the European Union's Horizon 2020 research and innovation programme, Marie Skłodowska-Curie grant agreement No 860715.

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Keywords: Cancer, Microenvironment and niche engineering

PP-183

A Novel Tissue Engineering Model for Urogynaecological Nerve Damage Using Electrospun Scaffolds

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INTRODUCTION: Due to the capability of electrospinning to fabricate artificial structures replicating form and/or function of natural tissues, these scaffolds could be increasingly utilised to model tissue damage and develop greater understanding of the mechanisms behind observed cellular effects. Peripheral nerve damage during pregnancy and labour can cause a range of life-affecting medical issues, including stress incontinence in up to 36% of first deliveries, but has no current gold standard treatment due to a lack of knowledge of the causal factors involved. The aim of this research was to apply a previously developed novel model, using electrospun scaffolds, to investigate the mechanisms behind urogynaecological nerve damage and possible tissue regeneration/repair.

METHODS: A range of polyurethane electrospun scaffolds was UV-Ozone sterilised and cell-seeded with human neurons (HN) and Schwann cells (HSC): HN; HSC; HN+HSC. Seeding densities of 5×10^4 cells/type/sample were used for 4, 7, 14, 28 days.

Assessed substrate parameters included: inter-fibre separation (IFS); fibre diameter (FD); surface roughness (SR); void fraction (VF); fibre orientation (FO); stiffness (YM). Cell-scaffold behaviour was examined for cellular growth, marker expression, process length/involvement/function and myelination potential (n=4 per sample/condition).

ANOVA statistical tests, with Tukey post hoc analysis, were performed to determine correlative relationships for cell-scaffold behaviour.

RESULTS: Key aspects of neural cell behaviour and functionality directly correlated to ranging and systematically altered scaffold properties, with determined relationships also seen in co-cultures. HN functionality, including axonal growth and alignment, was significantly affected by parameters in the horizontal plane (e.g. axon length by FD, $P < 0.001$ and $1/SR$, $P = 0.003$). HSC behaviour, including myelination potential, related to properties in the vertical scaffold plane (e.g. VF, $P = 0.044$, for cell orientation).

DISCUSSION & CONCLUSIONS: Neural cell interaction, behaviour and function were significantly affected by the differences and changes in scaffold properties. Correlating cellular effects to underlying substrate parameters, and systematic alteration to these conditions, allowed individual causes of negative cell responses and repair behaviour to be identified. Significant impacts to neural cell signalling and cell growth were amongst the observed consequences, as were indicators for potential causes of incontinence and reduced sensation. Further translating this information into the equivalent clinical and anatomical scenarios, through this novel model, can provide understanding of urogynaecological tissue damage, resulting symptoms and potential treatment options.

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Keywords: Interfaces - engineered, Trauma / surgery and rehabilitation

PP-184

Magnetic stimulation of TREK1 regulates stress signalling and neuronal connectivity in SH-SY5Y cells

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INTRODUCTION: The TREK1 potassium channel has multiple functions in the central nervous system, including neurotransmission, pain perception and neuroprotection and is therefore a potential pharmacological target for treating neurological disorders. TREK1 channels are known to be gated by a wide range of stimuli including mechanical force, making them amenable to mechano-activation using magnetic nanoparticles. Previous work has demonstrated that targeted mechano-activation of TREK1 using MNP and magnetic fields has useful applications in tissue engineering applications for regulating progenitor cell differentiation and bone formation.

METHODS: In this study the role of TREK1 mechano-activation in stress signalling, neuron differentiation and neuronal connectivity was investigated using the SH-SY5Y neuronal cell line. SH-SY5Y cells were first tagged with magnetic nanoparticles functionalised with an anti-TREK1 antibody allowing targeted activation of TREK1 channels. MNP tagged cells were then subjected to 1h sessions of magnetic force stimulation using a magnetic force bioreactor (MICA biosystems). Expression of stress response genes in response to magnetic stimulation was determined using qPCR and Nitrite production was measured using the Griess assay. The effect of TREK1 mechano-stimulation on neuronal differentiation and neuronal connectivity was then assessed using immunocytochemistry to stain for β III-Tubulin. Image analysis was performed using imageJ along with the Neurite Analyser plugin.

RESULTS: Our data showed that TREK1 can be mechanically activated using MNP and oscillating magnetic fields. Mechano-activation of TREK1 in this manner initiated expression of stress response genes including c-Myc and NF- κ B, along with Nitrite production, all of which are consistent with the cellular response to mechanical stress. This response did not affect cellular morphology or impair the neuronal differentiation capacity of SH-SY5Y cells when cultured in neuronal induction media, but did increase neurite number and decreased neurite branch length, suggesting TREK1 may regulate neuronal connectivity.

DISCUSSION & CONCLUSIONS: These results show that TREK1 is involved in the cellular stress response and activation of TREK channels promotes neuritogenesis without impairing neuronal differentiation or morphology. This approach could be developed towards an injectable cell therapy for engineering and promoting repair of neuronal tissue for the treatment of neurodegenerative disease, especially in conditions such as Parkinsons or Alzheimer's disease which require restoration of neuronal connectivity.

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Keywords: Nervous system (brain-central-peripheral / disorders), Biomechanics / biophysical stimuli and mechanotransduction

PP-185

Development of an "in vitro" model to study energy metabolism in Neural Stem derived cells, applications for Alzheimer Disease

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INTRODUCTION: The increase in life expectancy has led to a higher incidence of neurodegenerative disorders such as Alzheimer's disease (AD)¹. In the adult brain, neural stem cells (NSCs) can contribute to the repair processes by differentiating into neurons and astrocytes. NSC transplantation improves the behaviour of neurodegenerative mice models², as well as adult neurogenesis can provide new-born neurons that interact with brain network and improve spatial information processing and memory formation³. In AD, abnormalities in brain wiring are considered a key pathological framework of cognitive decline⁴. Our preliminary study aims to explore the potential differences between NSCs-derived neurons from healthy and AD mice in terms of neuronal network morphology. Furthermore, the study wants to provide a tool to investigate whether cell energy metabolism is modified, considering that bioenergetic failure can influence the regenerative potential of adult AD brain, an issue that is still ill explored.

METHODS: NSCs were isolated from subventricular zone of lateral ventricles of healthy and triple transgenic AD-develop mice at 6 months of age. NSCs selection was based on their ability to proliferate and to form 3D floating neurospheres. Isolated cells were amplified and characterised for their expression of stem-related markers. Subsequently, neuron and astrocyte differentiation protocols were set up. Morphological analyses were performed on healthy and AD NSC-derived neurons and the expressions of glutamate transporters and sodium/calcium exchangers (NCXs) were investigated.

RESULTS: NSCs isolated from both healthy and AD mice were strongly positive for Nestin and Sox2 stem markers and only a faint expression of NeuN and Gfap, markers related to neuron and astrocyte mature phenotype respectively, was detected. These data confirmed the purity of the cell cultures as well as the reliability of the protocols. Morphological investigations highlighted a different distribution of the neurite network between NSCs-derived neurons isolated from healthy and AD mice. Furthermore, healthy and AD mature cells expressed glutamate transporters essential not only for cellular transmission but also for energy production by their interplay with NCXs.

DISCUSSION & CONCLUSIONS: Preliminary data suggest that the pathological features of adult neurons from AD brain can be traced back to "stem level" and continue during differentiation. The different morphological organization of neurite network observed in NSCs-derive AD neurons could be involved in the alteration of the impulse transmission and plasticity. Further studies will be necessary to better clarify the consequence of dysregulated glutamate transporters as well the role played by astrocytes.

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Keywords: Nervous system (brain-central-peripheral / disorders), Stem cells - general

PP-186

Biohybrid synapse with functional biomembranes: from in vitro neuronal interface to biomimetic organic neuromorphic device

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INTRODUCTION: Neuronal loss and synaptic failure are common features of neurodegenerative disorders and often underlie the gradual motor and cognitive decline observed in patients. In this context, neuromorphic organic electrochemical transistors (OECTs), displaying biologically relevant electrical properties in simulating synaptic functions (e.g. short term plasticity, long term plasticity), have attracted significant interest providing simplified models to investigate brain's functionalities. Remarkably, such neuromorphic devices may pave the way toward the implementation of new class of neuroprostheses as therapeutic tools for the treatment of neurodegenerative disorders with significant potential in tissue engineering for in vitro and in vivo applications.

METHODS: In order to promote the seamless integration of such neuromorphic platforms and to bridge the mechanical mismatch between hard electronics and the much softer biological matter, several biomimetic approaches have been developed. In this scenario, supported lipid bilayers (SLBs), recapitulating the structure and the dynamicity of the biological plasma membrane, have proven to be a successful approach to trick cells to recognize artificial device as part of their native environment, thus securing tighter cell-chip interaction.

RESULTS: Here, in order to develop an in vitro biohybrid system able to improve the coupling between living cells and neuromorphic devices, SLBs were tested and optimized as platform for neuronal growth and then used to modulate OECT mediated synaptic plasticity.

To this purpose, SLBs were synthesized and functionalized to mediate neuronal behaviors at the cell-material interface. Here, the incorporation of negative charges within artificial membranes was able to mediate neuronal growth and neurites sprouting.

Then, SLBs were coupled with neuromorphic OECTs. Here, creating different conductive paths within the electrolytes, SLBs were able to influence the OECT behavior and stimulate short term plasticity.

DISCUSSION & CONCLUSIONS: Our findings may provide useful insights on how SLBs may be used to ensure the seamless integration of bioelectronic platforms within the biological world, paving the way toward the implementation of in vitro and in vivo neural platforms in tissue engineering.

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Keywords: Nervous system (brain-central-peripheral / disorders),

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Co-Culture of NGF cmRNA-transfected hMSCs and neuronal cells results in high levels of NGF and axonal outgrowth

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INTRODUCTION: Bone tissue engineering (BTE) efforts have progressed vastly in the past few decades, however research efforts of innervation for bone tissue engineering are limited. In bone, the peripheral nervous system detects sensory stimuli, yet innervation also helps regulate bone homeostasis. In research on innervation for tissue engineering, nerve growth factor (NGF) is often used to induce differentiation of neuronal cells in vitro. Generally, growth factors are associated with high dose requirements and high costs. Alternatively, transcript therapy using mRNA is proposed, where chemical modifications to mRNA (cmRNA) increase its stability and biocompatibility. In this research we propose a model for inducing innervation for BTE using a co-culture of NGF cmRNA-transfected hMSCs and PC-12 (Adrenal pheochromocytoma-derived) cells. We aim to induce differentiation of PC-12 cells to the neuronal phenotype through hMSC transfection with NGF cmRNA.

METHODS: PC-12 cells were seeded on a laminin-PDL-coated coverslip in a 24-wells plate. hMSCs were transfected in suspension using NGF-encoding cmRNA concentrations of 15.6 to 100 pg/cell encapsulated by Lipofectamine Messenger Max (Invitrogen) or NL37 (OzBiosciences). Transfected hMSCs were plated into transwells with 1.0 µm pores (CellQArt). Supernatant was collected 1-7 days post hMSC transfection for NGF determination by ELISA and PC-12 cells were fixed with 4% paraformaldehyde. Fixed PC-12 cells were stained with Phalloidin and anti-β-3-Tubulin and imaged. For secretome analysis a hMSC-PC-12 co-culture was performed in a 6-well plate using transwells with 1.0 µm pores (CellQArt). hMSCs were transfected with 62.5 pg/cell NGF cmRNA using NL37. Supernatant was collected 3 and 7 days after transfection. Liquid Chromatography Mass Spectrometry (LC-MS) was performed to analyse the secretome.

RESULTS: The optimal concentration and lipid vector to transfect hMSCs in co-culture were 62.5 pg cmRNA/cell and using NL37 as transfection reagent. This is based on the highest production of 11 ng/mL NGF. The secreted NGF was able to differentiate PC-12 cells into a neuronal phenotype and form axonal networks, as was observed after staining with anti-B3Tubulin and Phalloidin. LC-MS revealed that the secretome consisted of around 1500 proteins, including neuronal markers such as neurosecretory proteins, neurofilaments, Synapsin 2 and Beta-3-Tubulin.

DISCUSSION & CONCLUSIONS: Transfection of hMSCs with NGF cmRNA produced high amounts of functional NGF protein in co-culture and induced differentiation of PC-12 cells into the neuronal phenotype. Neurite length will be measured to quantify stainings. LC-MS analysis indicated the involvement of neurogenic pathways through neuronal markers. Future investigations include proteomics on cell lysates and pathway analysis.

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Keywords: Biologics and growth factors, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-188

Optimization of fibrin-agarose biomaterials for tissue engineering purposes

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INTRODUCTION: Fibrin-agarose (FA) biomaterials were previously used for the generation of bioartificial human corneas, skin, and other tissue substitutes (1). Although several types of agaroses have been fabricated to the date, the influence of the agarose type on the biocompatibility and biomechanical properties of FA has not been elucidated.

METHODS: FA hydrogels were generated with five types of agaroses at a final concentration of 0.05%, 0.1%, 0.2% and 0.3%. Human fibroblasts were immersed within to generate a stromal substitute. Each hydrogel was evaluated using a Instron 5943 biomechanical analyzer to determine the Young modulus, stress at fracture, strain at fracture and break load. To determine the biocompatibility of each type of agarose, live-dead analyses were carried out.

RESULTS: First, we found that the Young modulus of FA biomaterials was significantly higher than that of fibrin biomaterials devoid of agarose 0.29 ± 0.12 MPa vs. 0.17 ± 0.01 MPa, respectively. The same behavior was found for the strain at fracture ($261.10 \pm 76.48\%$ vs. 221.94 ± 23.19 for FA and fibrin alone, respectively), and for the break load (0.30 ± 0.14 N vs. 0.24 ± 0.08 N). However, differences were not statistically significant for the stress at fracture (0.50 ± 0.23 MPa vs. 0.49 ± 0.17 MPa). The highest values of these parameters corresponded to the FA tissues with agarose type F-D1LE, with proper results also obtained for LM agarose at the concentration of 0.05%. Then, we found that all agarose types and concentrations were biocompatible on human fibroblasts, with cell viability rates above 95% in all cases, especially at the lower concentrations of agarose.

DISCUSSION & CONCLUSIONS: These results confirm the high biocompatibility of FA biomaterials on human cells. The different biomechanical properties found when specific types and concentrations of agarose were used suggest that the biomechanical properties of FA tissues can be tuned and optimized for specific applications. Applications in skin, cornea, nerve and oral mucosa should use specific types and concentrations of agarose for clinical use.

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Keywords: Biofabrication, Biomaterials

PP-189

Basal membrane molecules expression by Wharton's jelly stem cell-derived microtissues

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INTRODUCTION: The umbilical cord has gained great attention due to its high content in Wharton's Jelly mesenchymal stem cells (WJMSC) with high potential in tissue engineering. It has been demonstrated that the basement membrane (BM) is a key structure implicated in stem cell niche that is present in different tissues and cells such as the epithelium of the skin and the Schwann cells of peripheral nerves (1). Microtissues (MT) can be generated in the laboratory from different types of cells, and this technique demonstrated its feasibility to generate scaffold-free 3D cellular-ECM structures with promising applications (2). The aim of the present study is to evaluate the expression of BM components by WJMSC-derived MT (WJMSC-MT).

METHODS: WJMSC were isolated from full-term umbilical cords and used to generate MT using 3.5% agarose microchips containing microwells of 400 µm of diameter. WJMSC-MT were cultured in standard culture conditions and harvested for immunohistochemical analyses after 4, 7, 14, 21 and 28 days of ex vivo development. Then, the synthesis of four major BM components (collagen type IV, laminin, perlecan and entactin-1) was evaluated in WJMSC-MT.

RESULTS: Histology revealed a clear maturation process and ECM remodelling within the MT over time. Perlecan and entactin-1 showed a homogeneous distribution, being positive at the ECM and cells. In contrast, collagen type IV and laminin reactions were restricted to the matrix-rich core of MT but with different localization in function of time. Interestingly, collagen type IV displayed a strong positive reaction with a membranous peripheral pattern, whereas laminin tends to be more diffuse or forming clusters.

DISCUSSION & CONCLUSIONS: This study demonstrated the ability of WJMSC-MT to synthesize major BM molecules during MT maturation. WJMSC-MTs could be a promising alternative to generate functional units of diverse bioartificial tissues, such as cornea, palate and neural tissue engineering, in which these ECM are essential to promote tissue regeneration.

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Keywords: Biofabrication, In vitro microenvironments

PP-190

Functional and electromyographical recovery profile following 15-mm peripheral nerve repair using nerve guides, acellular nerve matrices and autografts

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INTRODUCTION: The surgical treatment of critical nerve injuries is still a challenge. Nerve autograft remains as the gold standard technique, but new alternatives are still needed [1]. In this sense, decellularized peripheral nerve allografts (DPNAs) have emerged as a potential alternative because this method allows to generate complex and tissue-specific 3D matrices minimizing the immune response [2]. The aim of this study was to determine the efficacy of a novel chemical-enzymatic DPNAs (CE-DPNAs) in the repair of 15-mm sciatic nerve gap in rats in comparison with commercial nerve guides (NG group) (Neuragen®) and autograft technique (AUTO group).

METHODS: For CE-DPNAs, rat sciatic nerves were harvested and decellularized applying the new CE method (Triton X-100, SDS, SDC and an enzyme mix). Additionally, 15-mm sciatic nerve gap was created in adult Wistar rats and repaired with DPNAs, NG and AUTO (n=6 in each). The sensory, motor and electromyographic profiles were assessed after 15-weeks of surgery.

RESULTS: Functionally, the CE-DPNAs showed a higher degree of sensory and motor recovery than the Neuragen® commercial conduit. Interestingly, CE-DPNAs showed comparable sensory and motor recovery profile than AUTO group (p>0.05). The electromyography revealed signs of denervation and reinnervation in all groups. These results were more favorable -less denervation and more reinnervation- in AUTO group closely followed by DPNAs. Higher denervation and poor reinnervation was achieved in NG group.

DISCUSSION & CONCLUSIONS: This in vivo study revealed promising results with the use of the novel CE-DPNAs in the treatment of 15-mm nerve gap in rats. From the functional and electromyographic point of view CE-DPNAs were superior to conventional tubulization technique and closely comparable to autograft. However, it is still necessary to evaluate the nerve tissue regeneration across these graft at the histological and histomorphometrical level.

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Keywords: Nervous system (brain-central-peripheral / disorders), In vivo and animal models

PP-191

Generation of limbal crypts on decellularized sclerocorneal limbi for use in tissue engineering

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INTRODUCTION: The human sclerocorneal limbus is a complex structure hosting the limbal stem cells on specialized structures known as crypts allowing the normal homeostasis and proliferation of the limbal stem cells. Despite their relevance, crypts are very difficult to reproduce in vitro. One possible alternative is obtaining decellularized limbal scaffolds from native sclerocorneal limbi. However, decellularization typically alters the extracellular structure of decellularized organs, and novel biofabrication methods are in need to induce the generation of these structures on decellularized limbi.

METHODS: First, we obtained decellularized sclerocorneal limbi by applying decellularization protocols previously described by our research group [1,2]. Then, we designed a device able to generate crypts by using a 3D printer with PLA biomaterials. This device consisted in a mold with an elliptical pattern, where 11 needles of 500µm in diameter were inserted, with an inclination of approximately 30° towards the exterior of the curve, to reproduce the orientation of the native limbal crypts. This device was applied with a controlled pressure to generate crypt-like pathways on decellularized limbi. Then, corneal epithelial cells were cultured on these scaffolds, and the biocompatibility and efficacy of these new substitutes was evaluated by quantifying DNA release and by histological and histochemical analyses.

RESULTS: These studies showed that the use of the 3D-printed device applied to the decellularized scaffolds was able to generate detectable pathways resembling the native sclerocorneal limbal crypts. We also observed that corneal epithelial cells seeded on the scaffold were able to express typical corneal epithelial cell phenotypes from 7 days onwards. Cell viability was not affected by the scaffold, suggesting that these materials were biocompatible for use in tissue engineering.

DISCUSSION & CONCLUSIONS: The use of the 3D-printed crypts device, applied to the decellularized sclerocorneal limbus, can contribute to generate a more effective and biomimetic bioartificial sclerocorneal limbus for use in tissue engineering. Future studies in laboratory animals should determine the in vivo usefulness of this technology.

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Keywords: Eye, Biofabrication

PP-192

Spinal cord GAP-43 expression following peripheral nerve tubulization repair

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INTRODUCTION: Structural nerve injuries activate a complex regenerative process. During this process, upregulation of the growth-associated protein 43 (GAP-43) appeared to have a key role in neuritogenesis. Previous studies demonstrated that GAP-43 expression is a positive indicator of peripheral nerve (PN) regeneration (1). However, the role of GAP-43 in CNS following PN repair and regeneration is still poorly known. This study aimed to investigate the expression of GAP-43 in the spinal cord (SC) during early stages (10–30 days) of PN repair.

METHODS: In this study, 1-cm long defects were created in the sciatic nerve of adult Wistar rats. Defects were then repaired using NeuraGen collagen conduits. After 10, 20 or 30 days of surgery, animals were euthanized and the PN and SC were harvested for descriptive and quantitative histology (n=3 in each). Histological sections were stained with hematoxylin-eosin (HE) & MCOLL histochemical method, while GAP-43 and neurofilament (NF) proteins were identified by immunohistochemistry.

RESULTS: PN regeneration using conduit techniques showed an active PN regeneration process over time, indicated by a high positivity for GAP-43 and NF. Interestingly, GAP-43 expression showed an unexpected result in the SC. In fact, cells showed positive GAP-43 staining signal from day 20 of the surgical procedure onwards, and signal was specifically positive in the left (injury site) posterior columns of the white matter. Interestingly, a differential response was found between sensory and motor pathways. These differences were corroborated by the quantitative area fraction analysis that revealed significant differences in operated animals as compared to the control group (p<0.05).

DISCUSSION & CONCLUSIONS: Here, we demonstrated that an active process of PN regeneration may evoke early and differential changes in GAP-43 expression in the SC. As this protein is essential for cytoskeletal remodelling, our results suggest that this protein may play a crucial role in regeneration of the central nervous system after PN repair.

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Keywords: Nervous system (brain-central-peripheral / disorders), In vivo and animal models

PP-193

Ex vivo characterization of novel 3D-printed scaffolds for peripheral nerve tissue engineering applications

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INTRODUCTION: Peripheral nerves injuries (PNI) are prevalent in our population and often leads to different degrees of disability [1,2]. Currently, nerve autograft is the preferred treatment for severe PNI, but the drawbacks associated to this technique urge researchers to find novel therapeutic alternatives based on tissue engineering. The aim of this work was to perform a screening of the most suitable biomaterials to generate, by 3D technology, scaffolds potentially useful in neural tissue engineering.

METHODS: Here, we tested polycaprolactone (PCL), polylactic acid (PLA), thermoplastic polyurethane (FF), thermoplastic elastomer (FD) and gelatine methacrylate (GelMa) materials. Scaffolds were printed with a REG4Life 3D bioprinter (REGEMAT 3D S.L.) and subjected to scanning electron microscopy (SEM) and tensile tests (Instron, Model 5943). Furthermore, neuroblastoma cells (SK-N-AS cell line) were cultured within printed scaffold and the cell viability was determined by using WST-1 and Live/Dead assays after 72h of culture.

RESULTS: Thermoplastics scaffolds (PCL, PLA, FF and FD) exhibited better printability than GelMa (SEM). Tensile tests revealed higher stress at fracture and Young modulus values in PCL, FF, FD and PLA printed scaffolds as compared with GelMa. However, GelMa supported an optimal cell growth, being considerably more biocompatible than thermoplastic-based scaffolds.

DISCUSSION & CONCLUSIONS: Thermoplastic scaffolds resulted easy to handle with higher biomechanical properties than GelMa, as expected. However, GelMa scaffolds also showed promising structural, biomechanical, and printing properties, being considerably better in terms of ex vivo biocompatibility supporting an adequate growth of neural cell lines. However, future ex vivo and in vivo studies are still needed to determine the potential application of these scaffolds in peripheral nerve tissue engineering and repair.

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Keywords: Nervous system (brain-central-peripheral / disorders), In vivo and animal models

PP-194

Generation of bioartificial scleral limbi for use in tissue engineering of the eye surface

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INTRODUCTION: Treatment of corneal diseases with sclerocorneal deficiency is challenging, since corneal stem cells reside at this structure. Although several models of artificial corneas have been described, generation of a limbal substitute by tissue engineering is very complex and require further research. In the present work, we describe a method to generate a biological substitute of the scleral limbus by applying several decellularization methods on native scleral limbi.

METHODS: Sclerocorneal limbi were obtained from scleral rings. Then, limbi were decellularized using two different protocols: protocol 1 (P1) was based on the application of 1% SDS detergent, and protocol 2 (P2) consisted in a combination of SDS, Triton X-100, SDC, nucleases, and trypsin [2]. In both cases, an initial treatment with distilled water was applied to induce an osmotic shock on limbal cells. Evaluation of the efficiency of the decellularization process was determined by residual DNA quantification. Histological and histochemical analyses were performed for the study of extracellular matrix components preservation.

RESULTS: All protocols showed a decrease in DNA content, although only P2 fulfilled the requirements previously established for decellularized tissues (<50ng/mg dry weight). Histological analyses showed that both protocols preserved the components of the extracellular matrix, and collagen fibers and proteoglycans were properly preserved in limbi decellularized with P1 and P2.

DISCUSSION & CONCLUSIONS: These results suggest that the scleral limbus can be efficiently decellularized, especially when protocol P2 is applied. The complex structure of the scleral limbus probably requires the use of multi-step decellularization protocols combining chemical and enzymatic methods to ensure proper decellularization levels. The obtained scaffolds maintain the structure and composition of the extracellular matrix and could be a good candidate for subsequent recellularization for use in regenerative medicine.

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Keywords: Eye, Decellularised matrices

PP-195

Evaluation of epigenetic modifications in bioengineered human cornea substitutes generated by tissue engineering

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INTRODUCTION: Clinical translation of bioengineered human tissues implies a thorough histological, biological, molecular, and genetic evaluation as a requirement of the European Medicines Agency (1). Among others, cells subjected to 3D culture method in bioengineered tissue models could have epigenetic modifications that may alter the biological behavior of the artificial tissues.

METHODS: Human stromal cells were isolated from human biopsies using enzymatic methods. Cells were cultured on culture plates (2D cultures) and immersed within fibrin-agarose biomaterials resembling a stromal substitute (3D cultures). Two cancer cell lines were used as controls. Total DNA was isolated from 2D and 3D cultures and analyzed using the methylation array Illumina Infinium MethylationEPIC BeadChip, able to identify the methylation status of the promoter of most genes in each sample. Results were analyzed for the cell proliferation genes PCNA and MKI67, and for four relevant genes involved in pluripotential stemness commonly used to generate iPS cells (SOX2, OCT4, MYC, and KLF4).

RESULTS: Our analysis of promoter methylation revealed that 2 out of 4 probes analyzing the PCNA promoter were hypomethylated in control cells, but not in 2D and 3D cultures. For MKI67, the array contained 25 probes, and 3 of them were hypomethylated in controls, but not in 2D and 3D cultures. For the pluripotentiality genes, the 5 probes for SOX2 were found hypomethylated in all samples, without changes upon 3D culturing. For OCT4, we found that 4 of the 11 probes in the array became hypermethylated after 3D culturing. Of the 14 probes for KLF4, one became hypermethylated, and two, hypomethylated. Finally, of the 37 MYC probes, we only found hypermethylation of one probe after 3D culturing.

DISCUSSION & CONCLUSIONS: These results suggest that 3D culturing of human cells is not associated with a significant alteration of the epigenetic profile of relevant genes involved in gene proliferation and malignant transformation and support the safe use of 3D tissues made with fibrin-agarose biomaterials for cornea tissue engineering.

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REFERENCES: 1. Rico-Sanchez L, et al. J Tissue Eng Regen Med. 2019;13:2142-2154

Keywords: Eye, Scaling up & manufacturing

PP-196

Characterization of the Extracellular Matrix in Bioengineered Orthotypical and Heterotypical Human Corneas

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INTRODUCTION: Different clinically useful models of human bioartificial corneas were generated in the laboratory (1). Due to the slow proliferation rate of native cornea epithelial cells, several models of heterotypical corneas, in which the corneal epithelium is generated with alternative non-corneal cells (heterotypical artificial corneas -HAC-), were described. Although the differentiative potential of mesenchymal stem cells used in heterotypical corneas was demonstrated, the differentiation profile of the corneal stroma of HAC should be characterized. In this work, we have evaluated the fibrillar and non-fibrillar components of the stromal compartment of HAC both ex vivo and in vivo.

METHODS: HAC models were generated using fibrin-agarose hydrogels with keratocytes within and human umbilical cord Wharton's jelly stem cells WJSC on top to resemble the corneal epithelium. As controls, orthotypical artificial corneas (OAC) containing human cornea epithelial cells and keratocytes were generated. Both models were grafted at the cornea of New Zealand rabbits, and animals were followed-up for 12 months. Histochemical and immunohistochemical analyses were performed to determine the differentiation status of each cornea model.

RESULTS: The analysis of collagen fibers in bioartificial corneas as determined by picrosirius red staining showed higher synthesis of collagen in HAC models, as compared to OAC. Similarly, we found that non-fibrillar components such as proteoglycans and basement membrane markers were preferentially found in HAC models. Results also showed that the synthesis of relevant components of the extracellular matrix requires long periods of time, with differences between HAC and OAC samples. In vivo grafting significantly improved corneal stroma differentiation in both cornea models.

DISCUSSION & CONCLUSIONS: Our findings suggest a complete in vivo biointegration of both cornea models, with the synthesis of relevant components of the corneal stroma at the end of the follow-up time. WJSC demonstrated to be a potential alternative cell source to generate bioartificial human corneas and suggest that these cells could be potentially useful for the clinical treatment of corneal diseases when limbal stem cells are affected.

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REFERENCES: 1. Rico-Sánchez L, et al. J Tissue Eng Regen Med. 2019;13:2142-2154

Keywords: Eye, Biomaterials

PP-197

Implementation and evaluation of a self-oriented learning model for the acquisition of tissue-engineered competences by medical students

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INTRODUCTION: Traditional learning methodologies have been impacted by the introduction of new teaching approaches over the past few decades. In particular, the “self-oriented learning” format was proposed as a meaningful learning model for university student. This model previously showed a significant improvement of students perceptions and competences. The main objective of this model is to make the students the main protagonists of their own learning, with the generation of distinctive and elaborate learning tools such as the elaboration of a self-personalized notebook (1). However, there is no evidence of the effectiveness of this model in university teaching of Tissue Engineering (TE). In this study, we implemented and evaluated a self-oriented learning methodology in a TE course on medical students enrolled at the medical school of the University of Granada.

METHODS: Students enrolled in the subject of TE were confronted with practical sessions related to the biofabrication of artificial bone and muscle tissues (BMT). Students corresponding to the study group were directed using a self-discovery learning method in which students should design their own learning material and elaborate a HistoBook that will furtherly be the base of their study. In this self-elaborated notebook, students should include the main manufacturing methods related to these artificial human tissues and describe the main processes of BMT TE using graphical and/or digital resources. The first step was the identification and selection of the threshold concepts related to BMT. Then, students must elaborate a digital and/or graphical conceptual map of the different strategies to construct these tissues.

RESULTS: This preliminary study showed that students involved in this project satisfactorily identified more than 80% of the relevant threshold concepts related to TE and BMT biofabrication. The HistoBook document demonstrated to contain most of the key definitions and processes related to the generation of bioartificial BMT by tissue engineering. Students in charge of elaboration of the HistoBook showed their preference for this didactic model and would recommend this model to other students.

DISCUSSION & CONCLUSIONS: The self-oriented learning model demonstrated that students significantly acquired better transversal competences in TE and biofabrication of BMT, as compared to traditional-based learning models.

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REFERENCES: 1. Martin-Piedra MA, et al. Anat Sci Educ. 2022 Jan 24. doi: 10.1002/ase.2171. Epub ahead of print.

Keywords: Education,

PP-198

Full-thickness human skin equivalents behave like wounds

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INTRODUCTION: Full-thickness skin equivalents (FTSEs) are a promising approach for modeling human skin anatomy as well as studying wound healing in vitro. However, it is unclear how well FTSEs represent homeostatic (healthy/undamaged) skin or how much it resembles a wound-like state. We engineered FTSEs including dermal and epidermal compartments and compared them to human homeostatic skin and excision wounds.

METHODS: The FTSEs were constructed using a collagen-hydrogel containing primary human fibroblasts to form the dermal layer. Subsequently primary human keratinocytes were seeded onto the hydrogel to form an epidermal layer. Epidermal differentiation was initiated via air-liquid interface (ALI) culture and FTSEs were cultured for up to 28 days. For histological analysis, the FTSEs were fixed in 4% buffered formalin, embedded in paraffin, and sectioned. Paraffin sections were stained for epidermal differentiation and markers associated with wounding using immunohistofluorescence. The FTSEs sections were compared to homeostatic and wounded human skin.

RESULTS: Histological analysis revealed accurate differentiation and stratification of the epidermis similar to human skin, using the epidermal markers keratin 10, keratin 14 and loricrin. We observed that keratinocytes undergo progressive differentiation up to 12 days after ALI initiation, followed by a steep decline in epidermal thickness.

Most surprisingly, we discovered that all tested FTSEs were stained positive for proteins associated with wound healing like keratin 16. This was associated with activation of pathways involved in healing such as p-rpS6 and mitogenic protein cFOS. Finally, we found FTSEs enriched a marker of cellular senescence p21, which previous studies showed to be associated with wounds and the healing process. Finally, all these markers were present in high concentration in proximity to the excision wounds of human skin but not in homeostatic skin. The levels of markers were significantly different between homeostatic skin and excision wounds and homeostatic skin and FTSEs. Compared to the excision wounds, the FTSEs did not show a significant difference, suggesting a wound-like behavior of the FTSEs.

DISCUSSION & CONCLUSIONS: We demonstrated that FTSEs show the same molecular profile as human wounds expressing markers of cellular senescence, wounding-specific keratins, mitogens and signaling pathways. Overall, this to our knowledge is the first study to show that the commonly used model of homeostatic human skin in fact behaves much more like wounded than healthy human skin.

Keywords: Wound healing,

PP-199

A wound setting method for 3-layered *in vitro* skin models with intact artificial basal lamina to investigate reepithelialization processes

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INTRODUCTION: Human skin possesses a multilayered structure of the epidermis, dermis, and subcutis, providing a protective barrier against harmful external influences. Skin repair processes include the interaction of many different cell types, growth factors, and cytokines. Many wound healing studies are still performed in animal experiments; however, interspecies variations cause limited transferability of results to human systems. *In vitro* skin models, which mimic native human skin and enable crosstalk between the skin cell types, can be valuable alternative methods. In this study, a wound setting method for 3-layered skin models with an intact artificial basal lamina for modeling wound healing processes was established.

METHODS: The 3-layered skin models consisting of primary keratinocytes, fibroblasts, and mature adipocytes were constructed as previously described¹ with an artificial polymer membrane (0.4 µm pore size) between the epidermal and dermal layer. Skin models were wounded by 2 µm biopsy punches on both sides of the membrane, simulating a deep wound. The epidermis in the wound area was detached by enzymatic treatment and removed with tweezers. The course of wound healing was evaluated by monitoring keratinocyte migration into the wound area by tetrazolium dye (MTT) assay and transepithelial electrical resistance (TEER) measurements. The structure of wounded and non-wounded skin models was shown by hematoxylin-eosin staining compared to native skin. The enzyme-linked immunosorbent assay assessed the cytokine concentration of interleukin (IL)-6 and IL-8.

RESULTS: The established wound setting method enables to set defined wounds in the 3-layered skin model. Wound healing was successfully monitored by detecting reepithelialization of the skin models over 11 days after wounding. Epidermal wounds were closed after 4 days seen by MTT and proved by increasing TEER values. Cytokine release analysis of IL-6 and IL-8 could assess wound healing reaction.

DISCUSSION & CONCLUSIONS: In conclusion, the 3-layered skin model is suitable for wound healing studies, and integrating a subcutaneous layer provides an essential endocrine function for a more physiological *in vitro* wound healing model. Ongoing experiments continue to evaluate alternative non-animal-derived materials for the skin model.

ACKNOWLEDGEMENTS:

REFERENCES: F.F. Schmidt, S. Nowakowski, P.J. Kluger, Improvement of a Three-Layered *in vitro* Skin Model for Topical Application of Irritating Substances, Front. Bioeng. Biotechnol. 8 (2020) 1–11. <https://doi.org/10.3389/fbioe.2020.00388>.

Keywords: Wound healing, Other

PP-200

Degradation of a bovine collagen scaffold in a Human Bioengineered Skin transplanted on an athymic mouse model, tracked by LC-PRM.

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INTRODUCTION: Collagen is used widely in the manufacturing of dressing and tissue engineering either in a form of sponge, scaffold, or hydrogels. It is thus interesting to assess its degradation and replacement profile in vitro or in vivo. To assess this question, we have decided to use a bioengineered skin tissue grafted on an athymic mouse model.

METHODS: This bioengineered tissue consists of Human fibroblasts embedded in a bovine collagen type I hydrogel, overlaid with Human keratinocytes forming an epithelium. To analyze the degradation of the bovine collagen and capacity of the autologous fibroblasts to secrete human collagen, 1cm² discs of the bioengineered construct were grafted onto a full skin wound performed on athymic mice. Degradation of the bovine collagen scaffold and secretion of human collagen was monitored up to 6 months after grafting. To be able to specifically monitor the different types of collagen, an LC-PRM mass spectrometry method was developed to discriminate and quantify collagen type I and III of both origins. Standard Isotype Peptides matching with an amino-acid sequence specific of human and bovine collagen were synthesized, to specifically monitor the different types of collagens by using LC-PRM mass spectrometry method.

RESULTS: The grafts developed well on animals. Bovine collagen type I appeared to remodel drastically 2 weeks post-transplantation and this is confirmed at 6 months. In contrast, human fibroblasts persisted in the graft and consistently produced detectable levels of human collagen type I and III still at 6 months.

DISCUSSION & CONCLUSIONS: We therefore propose to use LC-PRM with the here described SIS-peptides to assess collagen remodeling of bioengineered tissue made of bovine collagen.

Keywords: Cell therapy, Wound healing

PP-201

Role of Scaffold Porosity in Treating Deep Skin Loss Wounds: A Finite Element Model

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INTRODUCTION: Deep wound treatments are currently limited in terms of healing outcomes, suffering from scarring and infection. During the second and third week of the wound healing, fibroblasts begin to express a myofibroblast phenotype resulting in linkages between cells and the extracellular matrix. These myofibroblasts can express smooth muscle actin and their presence can result in connective tissue compaction and wound contracture.

During the healing process, the wound edges contract, resulting in the reduction of the damaged area forming a scar tissue. In the case of deep wounds, this scar tissue causes the skin in the surrounding region to tighten which in turn can severely limit movement.

This tightening can spread to muscles and other tissues, leading to lasting loss of mobility. Overtime, scar tissue becomes stiff, making it difficult to manage with therapy and surgery, therefore early intervention is key. We propose that mitigating wound contracture by using scaffolds with tuned physical properties could result in favourable wound healing outcomes, reducing or eliminating the need for further surgery.

METHODS: The initial focus has been to develop a Finite element model of wound contracture. The simulation employs a neo-Hookean model to represent the material properties of the different layers of skin and the wound space. To compare wound contracture resulting from scaffold implantation in the wound space, a porous neo-Hookean material model was employed with a range 0.1 - 0.8 of solid fractions.

RESULTS: Increasing the solid fraction of the scaffold material mimics an increasing stiffness. The simulation results show that as the solid fraction increase, making the scaffold stiffer, it becomes more resistant to wound contractile forces, resulting in reduced stress, strain, and wound displacement. Natural skin tension (Langer lines) is also accounted for in the model via prescribed uniaxial contraction.

DISCUSSION & CONCLUSIONS: This simulation work is developing in parallel with the development of a skin tissue engineering scaffold targeted for treating large and deep wounds to regulate the wound contracture forces, resulting in reduced scarring. The finite element model will be updated with the addition of physical testing of scaffolds fabricated with a range of porosities to define the material model more accurately. This will lead to preclinical research involving small scale animal studies, to determine the ideal scaffold physical properties that result in reduced scarring outcomes.

Keywords: Skin, Wound healing

PP-202

A patient-centred microneedle patch for interstitial fluid sampling in scleroderma

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INTRODUCTION: We have developed hydrogel-coated microneedles to extract interstitial fluid (ISF) from human skin in a rapid and minimally invasive manner. This is an improvement over the current technique of suction-blisters, which is a painful and lengthy collection process requiring bulky vacuum equipment. Microneedles are short (~1mm) needles which cause little pain on insertion. These microneedles are intended to help diagnose and monitor patients with scleroderma – a life-limiting and under-diagnosed fibrotic condition that originates in the skin. Our device development was inspired by consultations with scleroderma patients.

METHODS: Puncture through human skin was proven by dyeing and microscopy. Force required for insertion was recorded in mechanical compression studies. Skin elasticity was quantified by atomic force microscopy (AFM) on tissue sections. Extracted ISF was analysed using weighing studies and protein assays.

RESULTS: Young's Modulus of skin is 55kPa in the epidermis and 5kPa in the dermis.

The average force required for insertion of a titanium microneedle array is 2N.

An average of 5µl ISF can be extracted in 20 minutes using microneedles on human skin, containing an average of 1mg total protein.

The biocompatible hydrogels methyl vinyl ether and alginate are both suitable super-swelling coating materials.

DISCUSSION & CONCLUSIONS: Various medical-grade materials were trialled for microneedle manufacture. Only titanium reliably penetrated the skin, indicating that metals are more suitable for this application than polymers (including Nylon, Polycarbonate and PLLA). This is partially due to the low Young's Modulus of human skin which enables elastic deformation around the microneedle. This finding informs the wider field of any microneedle device development.

The collection volume and speed of our microneedles exceed that of other sampling microneedles developed in the literature (e.g., Miller 2018's technology, which took 1-2 hours and required suction). Our short collection timeframe will likely increase patient compliance, and the minimally invasive technology will improve patient experience.

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Keywords: Polymers - natural / synthetic / responsive, Hydrogels and injectable systems

PP-203

Characterization of Extracellular Vesicles derived from preconditioned human Amniotic Membrane

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INTRODUCTION: The human amniotic membrane (hAM) has been recognized as promising source of therapeutic cells in tissue regeneration, namely human amniotic membrane mesenchymal stromal cells and human amniotic membrane epithelial cells, which have been attributed with stem cell characteristics. Procedures such as cell isolation and differentiation may lead to the risk of genomic instability and loss of intrinsic properties. A promising alternative is the usage of cell-derived bioactive factors released directly from the viable native hAM. Extracellular vesicles (EVs) act as cell-cell communicators by the intercellular transportation of lipids, proteins, DNA, RNA, and miRNA. It has been shown that EVs derived from several tissues exert regenerative potential, however, regarding the EVs derived from hAM in its native form, only little information is available. The aim of this study was to characterize EVs derived from preconditioned human amniotic membrane.

METHODS: To establish EV enrichment, supernatants from hAM punch biopsies of the placental amnion (P) and reflected amnion (RA) were collected at different time points and subjected to size exclusion chromatography (SEC), characterized for particle concentration and size distribution by nanoparticle tracking analysis (NTA) and fluorescence-triggered flow cytometry (FT-FC) for EV content, size, and composition.

RESULTS: For both P and RA, the number of extracellular vesicles and similar size particles increased after 48 h and 72 h, compared to 24 h of incubation, (for P samples, 24 h = 5.95×10^8 particles/mL, 48 h = 9.2×10^8 particles/mL, 72 h = 1.1×10^9 particles/mL), with particles smaller than 200 nm (mean = 140 nm, SD = 14; n = 3 biological donors).

FT-FC confirmed the presence of particles smaller than 200 nm (88 % of EVs). Positive signals for different tetraspanins (CD63, CD81, and CD9) demonstrated the presence of EVs. Of the characterized EV surface markers, CD81 was prominent in all samples (38 %, n = 2).

DISCUSSION & CONCLUSIONS: Our results showed information about conditioning time, size, and number of EVs secreted by hAM using SEC to isolate EVs. According to the number of particles detected, we consider a conditioning time of 72 h to continue with follow-up experiments. In our next step, we want to investigate the cargo of hAM-derived EVs pursuing a potential therapeutic approach to support tissue regeneration.

Keywords: Extracellular vesicles, Cell therapy

PP-204

Development of a femtosecond near-infrared laser-based cutting device for cell-assembled extracellular matrix based tissue engineered vascular graft manufacturing process

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INTRODUCTION: In Europe, the number of coronary artery bypass grafts and vascular access grafts for hemodialysis is increasing. Autologous blood vessels are often unavailable, and synthetic grafts are prone to infection and rejection. Our laboratory has developed a new generation of innovative Tissue-Engineered Vascular Grafts (TEVG) (allogeneic and non-living) inspired by the textile industry and adaptable to large-scale production and immediate availability by storage. Allogeneic sheets of a devitalized cell-assembled extracellular matrix (CAM) are cut into biological yarns with blades and woven to form a TEVG with physiological mechanical properties. However, this cutting process requires a long setting and remains challenging to operate under sterile conditions and to generate complex geometry. An interesting approach to processing biomaterials is laser ablation. Herein, we aim to develop a laser-based tissue-cutting device.

METHODS: This device was designed using a femtosecond laser in the near-infrared performing photoablation with microjoule-level pulse energy with a short duration of 264 femtoseconds. This laser setup has the advantage of high precision and reduces the thermal damage effect during the ablation process. We configured this laser setup to cut CAM sheets directly through a culture flask to maintain sterility and improve our current CAM ribbon manufacturing process. Using CAM in a hydrated or dry state, we determined the best settings to cut yarns under sterile conditions efficiently. The effect of this laser-cutting process on CAM structure damage was observed with scanning electron microscopy (SEM) and mechanical properties will be evaluated (Max tensile forces and percent strain at break).

RESULTS: Our results confirmed the ability of the laser to cut CAM biomaterial in both hydrated and dry conditions through a culture flask. To cut the hydrated CAM we needed more pulse energy resulting in more damage visible with a SEM. The lower pulse energy used on dry CAM offered a homogenous and more precise cut of our biomaterial. This laser photoablation process lightly modified CAM structure compared to ribbons generated with a blade. Using the laser on dry CAM we successfully generated complex geometry or micropatterning process.

DISCUSSION & CONCLUSIONS: This laser can cut at high precision while reducing contamination risks and tissue damage. The development of a laser-based device to cut our CAM biomaterials offers new possibilities for tissue biofabrication with complex geometries, micropatterning, or 3D model constructs.

Keywords: Biomaterials, Biofabrication

PP-205

Tissue Engineering principles translated for Cultured Meat

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INTRODUCTION: In order to mimic the complex morphology and hierarchical structure of living tissue, 3D bioprinting techniques that enable the precise positioning of cells and ECM materials have been explored in the field of tissue engineering for several years. Within the field of cellular agriculture (e.g. cultured meat), basic principles of tissue engineering are used to build up muscle and adipose tissue from diverse animal origins like bovine, porcine, chicken, or fish. A main focus is thereby laid on additive setup and material development.

METHODS: Given these facts, we have proven the benefits of a (dynamic) spheroid culture of primary bovine adipose-derived stem cells (bASCs), C2C12, and a chicken cell line (DF-1). Those spheroids were differentiated adipogenic or myogenic, and the success was demonstrated by protein staining.

RESULTS: Different eatable materials were evaluated to encapsulate those cells to build up lager constructs for cultured meat. Care was taken to ensure that the materials were cell compatible and exhibited nutrient values. After demonstrating that neither soy, or pea protein nor linseed, chia seed, and avocado seed extract is cell toxic, these additives were incorporated in polysaccharides. These materials mixtures were combined with single cells or spheroids and either set up manually or by bioprinting. All variations exhibited high viability and were able to maintain the desired functions. In addition, it was possible to construct a meat-like construct ($\sim 5 \text{ cm}^2$) and fry it without shrinking or destruction.

DISCUSSION & CONCLUSIONS: In summary, it was possible to show that it is useful to translate tissue engineering techniques for spheroid setup, cell encapsulation, hydrogel development, and bioprinting for the field of cultured meat.

Keywords: Biomaterials, In vitro microenvironments

PP-206

A three-dimensional glomerular millifluidic model to study lipid oxidation and extracellular vesicles turnover in idiopathic nephrotic syndrome

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INTRODUCTION: Idiopathic Nephrotic Syndrome (INS) is the most common glomerular-related childhood dysfunction, demanding an intense treatment with steroids and, in a minority of children resistant to immunosuppressants (SR), the requiring for kidney replacement therapy [1]. The complications of INS are linked to the lipid metabolism deregulation that can strongly affect the glomerular structural cells, the podocytes. Urinary extracellular vesicles (uEVs) have gained important recognition as potential diagnostic biomarkers in kidney disease, mirroring the pathophysiological state of cells along the nephron [2]. The generation of an innovative three-dimensional (3D) culture system, can mimic the evolution of the glomerular damage in INS, as well as, allow better evaluation of the EVs selectively secreted by different kidney cells.

METHODS: We develop a dynamic 3D model composed of human podocytes and endothelial glomerular cells, forming the glomerular filtration barrier (GFB). GFB-cells were cultured in a bioreactor and constantly perfused in closed circuit [3]. The dynamic glomerulus model was used to evaluate the role of steroid-resistant (SR) serum on the GFB. Cells apoptosis was evaluated by cytofluorimetric analysis. Actin cytoskeleton and slit diaphragm proteins modifications were traced by confocal microscopy. GFB-released extracellular vesicles (EVs) were isolated by ultracentrifugation and characterized by NTA. Lastly, lipid changes in GFB-cells and released EVs, were analysed by Mass spectrometry.

RESULTS: Through this model we could observe an increase in the barrier permeability to serum albumin when the dynamic glomerulus was treated with the SR serum, compared to the control treated with serum of healthy subjects, resembling aspects of INS glomerular damage. Focused on lipid metabolism, podocytes and their released EVs showed a distinctive profile in saturated and polyunsaturated fatty acids. The activation of the arachidonic acid (AA) synthesis pathway was strongly affected by SR-serum treatment, showing a possible role of FA desaturase 1 deregulation, involved in AA synthesis, in INS. Unbalance between anti- and pro-inflammatory fatty acids, associated with chronic kidney disease and inflammation, was observed in both podocytes and their released-EVs.

DISCUSSION & CONCLUSIONS: The 3D millifluidic dynamic glomerulus is a promising model to study glomerulopathies, demonstrating selective deregulation in lipid metabolism (like AA synthesis pathway) in SR-treated podocytes and showing the possible use of GFB-EVs lipid composition as INS biomarker.

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Keywords: Disease models, Extracellular vesicles

PP-207

Modelling and Prevention of Acute Kidney Injury through Ischemia and Reperfusion in a Combined Human Renal Proximal Tubule/Blood Vessel-on-a-Chip

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INTRODUCTION: Renal ischemia/reperfusion injury (rIRI) is one of the major causes of AKI. Although animal models are suitable for investigating systemic symptoms of AKI, they are limited in translatability. Human in vitro models are crucial in giving mechanistic insights into rIRI; however, they miss out on crucial aspects such as reperfusion injury and the multitissue aspect of AKI.

METHODS: We advanced the current renal proximal tubule-on-a-chip model to a coculture model with a perfused endothelial vessel separated by an extracellular matrix. The coculture was characterized for its three-dimensional structure, protein expression, and response to nephrotoxins. Then, rIRI was captured through control of oxygen levels, nutrient availability, and perfusion flow settings. Injury was quantified through morphologic assessment, caspase-3/7 activation, and cell viability.

RESULTS: The combination of low oxygen, reduced glucose, and interrupted flow was potent to disturb the proximal tubules. This effect was strongly amplified upon reperfusion. Endothelial vessels were less sensitive to the ischemia–reperfusion parameters. Adenosine treatment showed a protective effect on the disruption of the epithelium and on the caspase-3/7 activation.

DISCUSSION & CONCLUSIONS: A human in vitro rIRI model was developed using a coculture of a proximal tubule and blood vessel on-a-chip, which was used to characterize the renoprotective effect of adenosine. The robustness of the model and assays in combination with the throughput of the platform make it ideal to advance pathophysiological research and enable the development of novel therapeutic modalities.

ACKNOWLEDGEMENTS: This research project was supported by funding from Astellas Pharma, Inc.

Keywords: Kidney, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-208

Ameliorating impaired cardiac function in myocardial infarction using exosome-loaded gallic-acid-containing polyurethane scaffolds coated with decellularized extracellular matrix

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INTRODUCTION: Myocardial infarction (MI) refers to the condition which involves blockage of coronary arteries in the heart that results in the death of cardiomyocytes in the infarct zone, thereby compromising the contractile property of the heart. Cardiac patches are biocompatible materials that are utilized for providing mechanical support to the infarct portion of the heart. However, they face difficulty in controlling inflammation, attenuating oxidative stress, mechanical stability, and regeneration of damaged cardiomyocytes.

METHODS: In this study, we have synthesized an elastic polyurethane PUGA, with antioxidant phenolic compound gallic acid in its backbone, having cardioprotective properties and further coated it with caprine derived decellularized extracellular matrix (dECM). The scaffold was functionalized with exosomes isolated from adipose derived stem cells, which further aids in regeneration of damaged tissue. Successful synthesis of PUGA and dECM coating was checked through Fourier Transform Infrared Spectroscopy and Nuclear Magnetic Resonance. PUGA scaffolds were fabricated by cryogelation and tested for their morphology through scanning electron microscopy (SEM), antioxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay, and mechanical testing. Cytocompatibility was checked with H9C2 cardiomyoblasts and neonatal cardiomyocytes (nCMs) by MTT assay and immunostaining with specific markers. The physicochemical characterization, regenerative and antioxidant activity of the exosomes were also evaluated. Finally, the exosome-laden cardiac patch was tested in a rat model of myocardial infarction and improvement in cardiac function was assessed through echocardiography, histology and immunohistochemical staining.

RESULTS: The macroscopic architecture of the cryogel was visualized through SEM and elastic modulus was found to be ~300 KPa. The scaffold also attenuated oxidative stress and maintained significantly higher cell viability. The material was shown to be cytocompatible and showed expression of cardiac specific contractile and maturation markers by nCMs. A burst release pattern of the exosomes was observed (~75% within a week), which were able to attenuate oxidative stress and improved cell migration. After 8 weeks of implantation in an MI model, echocardiographic data showed significant recovery in PUGA-dECM + exosome group compared to MI group with respect to ejection fraction, fractional shortening, and cardiac output. Histological analysis showed decrease in fibrosis after application of exosome-laden patch. Finally, promotion of angiogenesis and reduction of oxidative stress was achieved, as depicted by expression of CD31 and 8-OHdG respectively. Expression of cardiac specific markers such as α -actinin, troponin I, and connexin 43 were also observed in the treatment groups.

DISCUSSION & CONCLUSIONS: Thus, the proposed combinatorial approach has potential to be utilized as a treatment strategy for tackling MI.

Keywords: Vascular systems / vascularisation and heart, Extracellular vesicles

PP-209

Implantation of a Tissue-Engineered Vascular Graft, Made of Cell-Assembled Extracellular Matrix and Produced with a Textile-Inspired Approach, in a Clinically Relevant Setting

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INTRODUCTION: Current commercial vascular grafts are made from synthetic materials that are stiff, not remodeled by cells, recognized as foreign bodies, a source of chronic inflammation, and prone to infections [1]. Our research group has developed a biological material synthesized in the laboratory by human or ovine dermal fibroblasts that we call Cell-Assembled extracellular Matrix (CAM) [2,3]. This study aims to demonstrate the short-term performance of a woven tissue-engineered vascular graft (TEVG) made of CAM in a clinically relevant setting.

METHODS: The TEVGs (5-cm-long, inner diameter = 4.1 mm) were woven on a circular loom using yarn made of ovine CAM sheets cultured for eight weeks. For each TEVG, one circumferential CAM thread was repeatedly inserted between a mobile and a fixed set of tensioned longitudinal ribbons of CAM (51 in total), creating a plain weave pattern. Woven TEVGs (n=4) were tested for suture retention, burst pressure, and transmural permeability.

Four TEVGs were grafted in anesthetized sheep as 5-cm-long interpositional carotid grafts. Doppler echography and angiography were realized four weeks post-implantation to show graft patency.

RESULTS: *In vitro* characterization of the woven TEBV revealed supraphysiological mechanical properties suitable for implantation with an average suture retention of 7.3 ± 2.1 N and a burst pressure of 4800 ± 760 mmHg (native ovine carotid = 2610 ± 860 mmHg). In addition, woven TEVG showed a low transmural water permeability of 15.7 ± 16 mL.min⁻¹.cm⁻², which is below the threshold of 50 mL.min⁻¹.cm⁻² often recommended for clinical use.

Surgical interpositional grafting of the woven allogeneic TEBV was realized without complication, and no blood leakage was observed through the wall after restoring the flow, indicating that the TEBV can be easily sutured to the native vessel using standard techniques and instruments. In addition, excellent patency, with no signs of graft dilation, was observed by echography and angiography after four weeks of implantation.

DISCUSSION & CONCLUSIONS: Finally, we successfully implanted for the first time a TEVG, made exclusively of CAM and produced using a textile-inspired approach, in a large animal that is clinically relevant both by its size and because it is an allogeneic setting.

ACKNOWLEDGEMENTS: This work was supported by the European Research Council and the French "Agence Nationale de la Recherche".

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Keywords: Cardiovascular, Biofabrication

PP-210

Bioactive Injectable Hydrogel for the *in Situ* Release of Surface-Modified Lipoplexes for Cardiac Regeneration via microRNA therapy

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INTRODUCTION: MicroRNAs (miRNAs) have emerged as a promising approach to favor cardiac regeneration after myocardial infarction (MI), inducing cardiac cells proliferation or direct reprogramming [1]. However, nanocarriers are required for safe and targeted miRNAs administration. Hyaluronic acid (HA)-based lipoplexes (LP) may allow active targeting, exploiting CD44 enhanced expression by cardiac fibroblasts (CFs) after MI [2].

Injectable hydrogels based on alginate dialdehyde (ADA) and gelatin (Gel) can be employed to allow *in situ* controlled release of miRNAs-loaded LPs. Incorporation of biochemical clues like thymosin β 4 (T- β 4), a peptide potentially suitable for cardiac repair, could further enhance gel bioactivity.

In this work, we developed novel HA/LPs for targeted miRNA delivery and double crosslinked hydrogels based on alginate (Alg), ADA and Gel for the local release of LPs and T- β 4.

METHODS: MiRNA-loaded LPs were prepared following a published protocol [3] and surface modified with HA, obtaining HA/LPs. HA/LPs physicochemical properties were investigated through Dynamic Light Scattering (DLS). MiRNA encapsulation efficacy and *in vitro* release were measured via Qubit™4-Fluorometer. Alg/ADA/Gel hydrogels were optimized varying polymers weight ratios and calcium ions concentration. Release studies of Cy5-siRNA-loaded HA/LPs from Alg/ADA/Gel hydrogels were investigated through UV-Vis spectroscopy. The effect of *in vitro* released T- β 4 from Alg/ADA/Gel hydrogels on adult human CFs (AHCfs) was evaluated through scratch test.

RESULTS: HA/LPs with 38 %w/w of HA showed the lowest hydrodynamic diameter (380 nm) and neutral Z-potential (-10 ± 1 mV), suitable for hydrogel loading. HA/LPs displayed long-term stability and high miRNA encapsulation efficiency (99%).

Double-crosslinked hydrogels were successfully optimized, combining chemical (ADA/Gel Schiff base) and physical crosslinking (Alg/calcium ions) to achieve cardiac-like viscoelastic properties. Complete and gradual release of a model fluorescent oligonucleotide, Cy5-siRNA, loaded in HA/LPs from Alg/ADA/Gel hydrogels was achieved in 14 days. T- β 4 release from hydrogels induced *in vitro* AHCfs migration, leading to complete wound closure after 24h.

DISCUSSION & CONCLUSIONS: A novel miRNA delivery system composed of HA-based LPs loaded in Alg/ADA/Gel-F hydrogels was developed. This multifunctional injectable system is able to mechanically sustain damaged myocardium and potentially provide cardiac regeneration through the targeted delivery of miRNAs and chemoattractant T- β 4.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 772168 (BIORECAR project).

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Keywords: Gene therapy, Drug delivery

PP-211

Role of Perfusion in the Generation of Stromal Vascular Fraction-Based Patches with Angiogenic Potential

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INTRODUCTION: Delivery of adult mesenchymal stromal cell (MSC) in three-dimensional (3D) constructs (patch-like fashion) represents a promising adjuvant angiogenic therapy in the treatment of chronic ischemia post-myocardial infarction (MI). In this context, adipose tissue-derived stromal vascular fraction (SVF) cells represent an appropriate cell source thanks to their heterogeneous cell system and well-proved high angiogenic potential (1). Culturing 3D-SVF-patches under unidirectional flow (3mL/min) has been shown to enrich the patches with extracellular matrix (ECM) components and pericyte-like SVF subpopulation, defined as CD45⁻CD146⁺CD34⁻ (hereafter CD146⁺cells). Upon *in vivo* ectopic implantation, perfusion-based SVF patches showed a higher cell-engraftment and angiogenesis compared to statically grown constructs (2). A better understanding of the role of perfusion in the generation of enriched-SVF patches is crucial for assessing their therapeutic potential. Therefore, we aimed (i) to identify the mechanisms behind the enrichment of CD146⁺cells in perfusion-cultured SVF patches and (ii) to evaluate the angiogenic potential of perfused and static-cultured SVF patches in an *in vivo* orthotopic model.

METHODS: Human adipose tissue-derived SVF cells were cultured on 3D-collagen-sponges (8mm diameter-3mm thickness), either in unidirectional perfusion-based bioreactors or in static condition for 5 days. Flow cytometry, immunofluorescence and immunoblot analyses were performed. The *in vivo* angiogenic potential was assessed at 28 days on healthy nude rat hearts.

RESULTS: Although the exposure of static-cultured SVF patches to the secretome released under perfusion did not increase the ECM components and CD146⁺cells, their enrichment in perfused-SVF patches has been related to the flow-rate in a specific pattern. Among the mechano-related signaling, the ERK1/2 pathway resulted activated in perfusion-engineered patches. Interestingly, the inhibition of the ERK1/2 pathway under perfusion impaired the enrichment of CD146⁺cells, while it did not affect the other SVF subpopulations. Upon epicardial implantation in healthy hearts, perfusion-based SVF patches showed significantly higher levels of engraftment. Interestingly, perfusion-cultured SVF patches displayed increased vascularization potential within the patch itself (intrinsic) and also around its border with the healthy myocardium (extrinsic).

DISCUSSION & CONCLUSIONS: This study revealed a crucial role of perfusion-culture in generating SVF-patches enriched with ECM components and pericytes-like cells (CD146⁺cells). Furthermore, we provide evidences of the positive effect of the perfusion on the angiogenic potential of SVF patches in a healthy heart. To fully assess the repair/angiogenic potential of the perfusion-based SVF-patches further *in vivo* investigations in a chronic ischemic heart model are required.

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REFERENCES: 1.Ramakrishnan et Boyd, 2018; 2.Cerino et al, 2016.

Keywords: Biomaterials, Cell therapy

PP-212

Fabrication of multilayered tissue-engineered vascular grafts with aligned smooth muscle and endothelial cells

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INTRODUCTION: Tissue-engineered vascular grafts (TEVGs) are advancing rapidly to replace small blood vessels, yet no clinically successful TEVGs have been reported, and many that have been developed lack the mechanical properties and structure of native blood vessels (NBVs) [1]. In this study, we aim to fabricate a multilayer TEVG to mimic the structure, mechanical strength, and cellular orientation of NBVs.

METHODS: TEVGs comprised of two layers, a hollow tube of electrospun polycaprolactone (PCL) surrounded by a layer of cast gelatin methacrylate (GelMA), were fabricated and characterized. The PCL increases the mechanical strength of the graft, while the GelMA layer prevents leakage and provides a scaffold for smooth muscle cells (SMCs). To measure the mechanical properties of the TEVGs, burst pressure, tensile extension, and suture retention tests were performed. Endothelial cells (ECs) and SMCs were seeded in the TEVG and cultured for seven days before confocal imaging to observe their morphology and alignment.

RESULTS: Assessment of the mechanical properties of the TEVGs showed that they have comparable burst pressure, suture retention, and circumferential tensile strength to NBVs. After 7 days of culture in static conditions, confocal images showed that ECs formed a confluent monolayer, with stretched, connected, and axially aligned morphology. SMCs were likewise stretched and circumferentially aligned around the lumen of the TEVG.

DISCUSSION & CONCLUSIONS: The similarity of the mechanical properties between the TEVG and NBVs is essential for the stability and safety of the graft in the body. The full endothelium in the lumen is important as the ECs play a critical role in preventing thrombosis and in regulation of nutrient transport to surrounding cells [2]. The circumferential alignment of SMCs is related to their contractility, which is essential for functional blood vessels to contract and dilate [3].

A facile technique has been developed to fabricate multilayer TEVGs with similar mechanical properties to NBVs. It also promotes axial alignment of ECs and circumferential alignment of SMCs in static culture. These findings shorten and simplify the process required to fabricate a vascular graft with aligned ECs and SMCs, without needing a perfusion bioreactor

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Keywords: Vascular systems / vascularisation and heart, Biofabrication

PP-213

Induced Pluripotent Stem Cell (iPSC)-Derived Secretome as a Cell-Free Therapy to Improve Angiogenic Function in Hypoxia-Injured iPSC-Endothelial Cells

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INTRODUCTION: Acute myocardial infarction (MI) is a leading cause of cardiac disability worldwide. Post-MI, the human heart undergoes maladaptive remodelling due to the inability of differentiated cardiovascular cells to repair themselves sufficiently and regain pre-infarct functional status. Many cardiac disorders are characterised by endothelial cell (EC) pathophysiology, as ECs modulate blood vessel barrier function, line their luminal surfaces and regulate oxygen and micronutrient transport to other cells. Consequences of EC pathophysiology often manifest as heart failure in surviving patients, an irreversible cardiovascular disease with no cure. For decades, stem cells have been studied due to their regenerative potential, however, stem cell clinical trials failed to yield desirable improvements. Now, the focus has shifted towards cell-free regenerative therapies. Therefore, we investigated a novel cell-free strategy, employing stem cell secretome which is composed of different cell-secreted components such as growth factors, miRNAs and exosomes. These secretome-derived factors are used in intercellular communication of anti-apoptotic, proliferative and angiogenic signals, thus increasing tissue regeneration. There is limited research investigating induced pluripotent stem cell (iPSC) secretome's effect on damaged cardiovascular cells. Therefore, the purpose of this study is to evaluate the effectiveness of iPSC secretome in the rescue of iPSC-derived ECs after hypoxic injury. We hypothesize that iPSC secretome will significantly increase the injured iPSC-endothelial metabolic activity, viability and angiogenic properties, improving angiogenesis *in vitro*.

METHODS: Initially, pluripotency of iPSCs generated from peripheral blood was confirmed via immunostaining. Subsequently differentiated iPSC-ECs were injured via hypoxia (0% oxygen, 24h). Moreover, undifferentiated iPSCs were incubated in serum-free basal media, at 37°C (24h); iPSC secretome was then collected, filtered and stored at -20°C until further use. Following secretome administration, tube formation was analysed (16h). After secretome incubation (24h), cell viability, metabolic activity, tube formation and cell migration were assessed.

RESULTS: iPSC-ECs displayed EC morphology and expressed endothelial markers (CD31, CD34). Under hypoxic conditions, untreated iPSC-ECs had lower cell viability and tube formation (evaluated as branch number and length; average number of nodes). However, iPSC secretome rescued iPSC-EC response to injury, yielding comparable cell viability and tube formation as normoxic iPSC-ECs.

DISCUSSION & CONCLUSIONS: This preliminary data suggests that our iPSC-ECs are endothelial-like cells. Also, iPSC secretome increased the injured iPSC-EC viability and tube formation capacities. We hope this preliminary knowledge will be a stepping stone into the development of novel therapeutics for vascular damage following MI.

Keywords: Induced pluripotent stem cells, Cell therapy

PP-214

Artificial small-diameter blood vessel with enhanced endothelialisation properties

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INTRODUCTION: Patients suffering from occlusive arterial disease following atherosclerosis are in desperate need of a reliable means to replace the defective vessels at sites of the occlusion. Insufficient perfusion from small-diameter blood vessels underpins much of the dysfunction in tissues and organs seen in chronic conditions (hypertension, obesity, and diabetes mellitus). Surgical grafts for smaller vessels lack long term patency while synthetic vessels are prone to develop acute thrombosis and intimal hyperplasia. Consequently, to achieve acceptable long-term patency in small calibre prosthetic grafts, not only antithrombogenicity but also excellent healing property are also necessary.

Therefore, we investigated an innovative approach to trigger the self-healing properties by enhancing the pro-angiogenic activity of vascular endothelial growth factor (VEGF), a powerful blood vessel-forming protein produced at sites of tissue ischaemia. Hence, along with the development of a synthetic small calibre blood vessel, this project seeks to supply the essential co-factor to VEGF, a heparan sulphate (HS) that binds and maximally amplifies the effects of the VEGF. Herein, a potent HS variant that we have isolated and characterized based on its targeted-affinity to VEGF is proposed to be incorporated into the manufacturing of the vascular prosthesis.

METHODS: Artificial vascular prostheses of small diameter were obtained through electrospinning technique. The incorporation of the pro-angiogenic HS was tested with a variety of polymers and co-polymers of medical grade. The artificial vascular prostheses were designed to mimic small arterial vessels topography and microstructure. The physical, biochemical, and biological properties were assessed to determine the vascular functionality and graft potential.

RESULTS: All composite constructs showed good incorporation of HS in their structure observed in a dose-dependent manner. Furthermore, HS increased the surface hydrophilicity of the material (water contact angle), while it strengthened the mechanical resistance (tensile stress and hysteresis) in all groups. HS entrapped in the polymer fibrous structure preserved its bioactivities with high VEGF retention, protection, and sustained release in a kinetics study (ELISA). Finally, the addition of HS significantly increased the adhesion, survival, and proliferation of human endothelial cells on the luminal surface of the vascular graft in static and flow culture in vitro (bioreactor).

DISCUSSION & CONCLUSIONS: The electrospun manufacturing of tubular materials incorporating pro-angiogenic HS has demonstrated promising outcomes in terms of reproducing native-like topography, microstructure, elastic properties, patency, blood compatibility, and strong potential in the establishment of a functional endothelium leading the way for successful self-regenerative potential in clinical applications.

Keywords: Biomaterials, Vascular systems / vascularisation and heart

PP-215

Dynamics of sprouting vascular networks: from growth monitoring to general growth rules

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INTRODUCTION: Bead-sprouting assays remain the gold standard in angiogenesis research. The bead method relies on the assessment of the endothelial sprouts emerging from a cell-coated microbead suspended in an external ECM-mimicking hydrogel as a way of quantifying the angiogenic potential of the given type of cells, ECM, or the presence of growth factors. Here, we use the sprouting beads to study the general growth rules governing the development of microvascular networks. Our approach relies on day-by-day confocal imaging of tens of the sprouting networks and their morphometric analysis using custom-developed high-throughput image analysis tools. We track the evolution of the statistical distributions of branching angles, segment lengths, numbers of branch generations, etc..

METHODS: Polystyrene microbeads coated with GFP-tagged HUVECs were embedded in 2.5 mg/mL fibrin hydrogel. The beads were seeded in 24 well plate, one bead per well, each positioned centrally in the well. We acquired confocal images at 24h time intervals over the period of 14 days to cover all stages of the angiogenic morphogenesis. Our approach goes beyond current standards which typically consists of a single time-point analysis (e.g., day 7 or day 14). We also develop an automated Python-based image processing tool which extracts the morphology of the sprouting networks and produces a database of measured metrics.

RESULTS: We reveal that the evolution of the sprouting networks proceeds via three stages: (i) the 'idle' stage, (ii) the sprouting stage, (iii) the maturation stage. The segment lengths are distributed exponentially which supports a picture of a stochastically branching network where, in particular, the characteristic length does not depend on the VEGF concentration. However, at higher VEGF, we observe sprouting of a larger number of primary branches and their more active bifurcations resulting in overall more complex networks. The average values of the branching angle vary in the range of 65-75 degrees, i.e., close to $(2\pi/5) \times 180 = 72$ degrees characteristic of the Laplacian-growth models, overall suggesting that the tips follow the highest local nutrient/oxygen concentration.

DISCUSSION & CONCLUSIONS: Our detailed analysis is a step towards engineering of optimally vascularized tissues. In particular, we find that the dynamics of growth becomes saturated at a certain VEGF concentration and in such a case the networks can span up to 2 mm in diameter. Vascularization of larger constructs would therefore require multiple dispersed beads to serve as the angiogenic 'seeds'.

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Keywords: Vascular systems / vascularisation and heart, Microenvironment and niche engineering

PP-216

The impact of flexible polycaprolactone scaffolds with hierarchical multiscale porosity on dendritic cells

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INTRODUCTION: Bone tissue engineering (BTE) works on utilizing cells, bioactive molecules and scaffolds into therapies that facilitate tissue regeneration and restore function. Bone is a highly dynamic organ and replacing such an intricate construct with cognate materials is challenging. One biomaterial used in BTE due to its mechanical properties and biodegradability is polycaprolactone (PCL). PCL physiochemical properties can be modified via several fabrication and functionalization methods. However, altering the architectural properties of scaffolds might affect immune host responses after implantation, and thus impact their regeneration potential. Dendritic cells (DC) are crucial for bridging innate and adaptive immunity, foreign body reactions and play an important role in tissue regeneration processes.

The aim was to investigate how our highly porous PCL scaffolds affect monocyte differentiation into dendritic cells and the monocyte derived dendritic cell (moDC) activation.

METHODS: To mimic bone structure, PCL ink was 3D-printed at room temperature in an ethanol bath to attain hierarchical multiscale porosity. Human CD14⁺ monocytes were isolated from blood through negative selection, and were seeded onto PCL scaffolds (250.000 cells/cm²). Monocytes were cultured in tissue culture plates and served as 2-dimension (2D) control. Differentiation into moDC was stimulated using GM-CSF and IL-4. Pro- and anti-inflammatory cytokines were evaluated at gene level, C-C motif chemokine 22 (CCL22) release was measured using ELISA and moDC differentiation and activation markers were evaluated using flow cytometry

RESULTS: Monocytes grown on PCL expressed higher levels of genes IL6, IL10, TNF, CTLA4 and CCR2, while ARG2 transcripts were increased in moDC on PCL. Differentiation into moDC on PCL scaffolds was comparable to 2D as assessed by flow cytometry for CD14 expression. CCL22 release was reduced in PCL compared to 2D for both monocytes and moDC.

DISCUSSION & CONCLUSIONS: These data suggest our scaffolds retain properties that allow for differentiation into moDC, however CCL22 release was observed to be reduced in our PCL scaffold. This warrants further investigation to understand how these pathways might be affected and how this impacts the bone regeneration.

These findings may help guide the design of scaffolds to promote immune-mediated tissue regeneration.

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Keywords: 3D printing and bioprinting, Polymers - natural / synthetic / responsive

PP-217

The role of monocyte-derived macrophages as endothelial progenitors in endothelial injury

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INTRODUCTION: Endothelialization of implanted cardiovascular devices is the golden standard for obtaining a hemocompatible surface, yet it has not shown a complete success. Typically, the pool of cells contributing to endothelialization is thought to be limited to mature ECs, EPCs, and small population of other stem cells. Recently, monocytes have been reported as an additional source of endothelial-like cells in implanted vascular grafts. However, to what extent these cells can exert endothelial functions and the exact mechanism of how they integrate within a newly formed/injured endothelium is yet to be explored.

In this work we investigate to what extent monocyte-derived macrophages can functionally integrate in an injured endothelium and whether this is dependent on biochemical priming.

METHODS: A wound healing model was established in which an endothelial monolayer is co-cultured in direct contact with human monocyte-derived macrophages that are primed into various polarization states (i.e., M1, M2a & M2c). The extent of monocyte-to-endothelial differentiation and functional integration into the endothelium was characterized by live-cell imaging, immunofluorescence, and flow cytometry.

RESULTS: Scratch closure was faster in the co-cultures containing M2a and M2c macrophages compared to M1. Looking closer into all co-culture conditions, physical contact via VE-cadherin was noticed between endothelial cells and M2c macrophages. Flow cytometry results showed that M1, M2a, M2c had an initial increase in macrophage markers CD80, CD206 and CD163, respectively. This was stabilized after 8 days in culture with no further increase. On the other hand, there was a gradual increase in endothelial marker CD144 mainly in M2c macrophages after 8 days in culture.

DISCUSSION & CONCLUSIONS: The results of this study reveal a new aspect of endothelial regeneration, namely through macrophage differentiation. Currently we are exploring whether the shift of M2c macrophages towards the endothelial phenotype is due to paracrine signaling. This shall steer biomaterial design choices (e.g., surface bioactivation) for implanted cardiovascular devices.

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Keywords: Immunity / immunomodulation / macrophage, Cardiovascular

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Local immune response to stem cell-based bone regeneration in a rat osteoporotic model

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INTRODUCTION: Bone tissue engineering aims to treat clinically challenging bone defects by combining biomaterial scaffolds and bone marrow mesenchymal stem cells (BMSC) as bone tissue engineered (BTE) constructs. The immune response after implanting BTE constructs plays a critical role in the success of the regeneration process. This is particularly important in the context of compromised healing, such as in bone pathologies like osteoporosis. Recently, the immune system has been shown to play a critical role in the occurrence and development of osteoporosis. Therefore, this study aimed to characterize the immune cells responding to BTE constructs in an osteoporotic rat model, and consequently understand how that impacts the regeneration process.

METHODS: Bi-calcium phosphate scaffold granules (BCP) alone or seeded with BMSC (BCP+BMSC) were implanted in calvaria defects of ovariectomized rats. The scaffolds were harvested after two weeks, and a single-cell suspension was obtained through rounds of optimized enzymatic and mechanical digestion. Isolated cells were analyzed using a multiparametric flow cytometry panel for a broad landscaping of infiltrating immune cell populations. Additionally, mRNA expression of immune-related (CSF1, TGFβ, TNFα, IL-10) and osteogenesis-related genes (SPP1, RUNX2, COL1A1, ALP) from harvested scaffolds were measured with RT-PCR.

RESULTS: Scaffold-associated immune cells in both groups presented a higher number of T and B cells in comparison with other immune cell types, while macrophages and neutrophils were below 20% of total CD45+ cells. The composition of infiltrating cells was comparable between both groups except for a slightly higher proportion of B cells in BCP+BMSC. Furthermore, osteogenic-related genes had a higher expression in BCP+BMSC, while immune-related genes had lower expression in this group indicating immunomodulation that promoted the expression of bone regeneration markers.

DISCUSSION & CONCLUSIONS: This modulation suggests a role played by the immune cells to enhance the efficiency of BMSC therapy. It can be postulated that the adaptive immune system plays a major role in regeneration in osteoporosis, particularly the B cells when considering stem cell-based regeneration. To our knowledge, this is the first report of immunophenotyping for single cells isolated from BTE constructs, and its relation to BMSC osteogenic potential as a therapy for regeneration in osteoporotic animals. This serves as a basis to understand the intricate immune regulation of regeneration in compromised healing.

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Keywords: Biomaterials, In vivo and animal models

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Precision vehicles for combined targeted drug delivery and cellular reprogramming

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INTRODUCTION: Hybrid multifunctional nanosystems constitute a promising strategy for modulating cellular responses in complex processes such as resolving inflammation or tissue regeneration.

Magnetic polymeric micelles(MPMs) concurrently incorporate hydrophobic(core) and hydrophilic(shell) cargos boosted with the possibility of magnetic power offered by superparamagnetic iron oxide nanoparticles(SPIONs) for untethered control, tracking, imaging, and intracellular delivery via magnetofection.

We propose the assembly of MPMs from palmitic acid-grafted-chitosan(PA-g-CS) and SPIONs, aiming at the miR-based modulation of macrophage functions to overcome persistent inflammatory events. Nile Red(NR) and Fluorescein Sodium(FL) were used as hydrophobic and hydrophilic model drugs, respectively. A miR antagonist for pro-inflammatory miR-155 was also loaded in the CS segment. The transport of RNA species remains an unmet challenge, despite the emerging promise of RNA-based therapeutics. MPMs are expected to enable the spatial distribution of distinct therapeutic agents with complementary actions for targeted guidance of healing events.

METHODS: MPMs were assembled from PA-g-CS and SPIONs, the first being prepared by carbodiimide coupling chemistry. Pyrene was used to determine the amphiphile critical micelle concentration(CMC). We explored SPION:PA-g-CS mass ratios of 1:2 and 1:5 to derive MPMs with the most appropriate physico-chemical characteristics. MPMs were produced by ultrasonication. NR and FL were used to evaluate the carrier-loading potential. L929 and THP1 cells were cultured with various MPMs concentrations at each mass ratio for viability, uptake, and functional assessments by qPCR, immunochemistry, and flow cytometry.

Magnetofection was performed using a static magnetic field (MagnefectNano, 350mT, 20min).

RESULTS: The PA-g-CS polymer self-assembles forming micelles at $>254 \pm 6$ ug/mL(CMC). MPMs present a spherical morphology with core-incorporated SPIONs, low polydispersity, and similar hydrodynamic diameter (aprox. 354 nm) and surface charge (+22 mV) for the studied SPIONs:polymer mass ratios. The SPIONs loading efficiency(LE) determined by ICP was higher for 1:2 (66%) compared to 1:5 (31%). Interestingly, the SPIONs loading influences the NR loading with lower LE in the 1:2 formulation (38% vs 63% for 1:5). On the shell, the FL showed the lowest LE: 5.2% (1:2) and 23% (1:5) likely due to its small size and easy leakage from the shell.

MPM did not compromise cell viability and the accumulation of NR-loaded-MPM within cells suggests MPMs feasibility as intracellular carriers. Varying the miR:polymer mass ratio it is possible to bind miR to MPMs, whose impact is being addressed on macrophage polarization mechanisms.

DISCUSSION & CONCLUSIONS: MPMs were successfully produced and show potential as multi-load delivery nanoplatforms for RNA-based therapeutics targeting immune cells.

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Keywords: Cell therapy,

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Improved absorption capacity of allografts for antibiotic loading

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INTRODUCTION: Treatment of bone defects and infections still poses a challenge. Grafting materials are used to fill defects, which can ideally be loaded with antibiotics if necessary. An important aspect is the absorption capacity and the retention of the antibiotic in the material.

The aim of the study was to compare different allografts with regard to the absorption and release of antibiotics. A specially designed high-absorbency, high-surface area carrier graft composed of demineralized cortical fibres and granulated cancellous bone was compared to different human bone allografts.

METHODS: Investigated carrier grafts included three groups of fibrous allografts (rehydration rate: 2.7, 4 and 8 ml/g), demineralized bone matrix (DBM), cortical granules, mineralized cancellous bone and demineralized cancellous bone (all manufactured by the DIZG, Germany). The absorbent capacity was assessed after rehydration with phosphate buffered saline (PBS, final volume of 0.4 cc).

Rehydrated samples were centrifuged in a sieve to determine total and matrix-bound absorbent capacities separately. Rehydration with gentamicin (40 mg/ml, final volume of 0.4 cc) was performed to determine the elution kinetics. Loaded grafts were eluted in PBS and elution samples were taken over a period of 21 days (Gentamicin quantification: GENT2, Roche GmbH). The antimicrobial activity of the eluted samples was demonstrated by a zone of inhibition (ZOI) test with *S. aureus*.

RESULTS: The greatest tissue matrix absorption capacity was observed in the fibrous allograft, while mineralized cancellous bone exhibited the lowest matrix-bound absorbent capacity.

The initial release of gentamicin was highest from fibrous allografts with a rehydration rate of 8 ml/g. A prolonged release for three days was observed for allografts with a rehydration rate of 2.7 and 4 ml/g with significantly higher amounts compared to the other grafts. Absorption capacity and release kinetics were only marginally affected by the incubation time varying from 5–30 min.

The largest ZOI occurred for the 1-hour eluted samples, independent of the graft type. The prolonged release from the fibrous grafts was also confirmed by the prolonged antimicrobial activity compared to the samples from the other grafts.

DISCUSSION & CONCLUSIONS: The improved absorption capacity of the fibrous allografts results in a prolonged release of the antibiotic. The retention of the fluid makes the handling easy. It can therefore serve as a suitable carrier graft as it provides a convenient way to keep fluids such as antibiotics at their intended destination and allows a prolonged release for septic orthopaedic indications.

Keywords: Biomaterials, Drug delivery

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Antimicrobial coating for orthopedic implants using covalent immobilization of peptides

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INTRODUCTION: Despite recent progress in the strategies of antimicrobial coatings for orthopedic implants, developing a technique to selectively eliminate bacterial attachment without affecting the osteoblast cell adhesion and proliferation remains a research precedence. Up to now, coating strategies based on antimicrobial peptides (AMPs) can substantially tackle infectious problems where bioinspired peptides can be immobilized through covalent attachment to the implant surfaces. The covalent immobilization allows long-term activity and sustained release of AMPs to inhibit bacterial colonization and subsequent biofilm formation. However, this approach still requires higher antimicrobial activity, resistance to enzymatic degradation, long stability, and lower cost of industrial scale.

METHODS: The present study aims to propose a reliable technique for antimicrobial peptide grafting on the implant surfaces using stimuli responsive strategies to improve the antimicrobial activity and stability of the coating as well as selectively improve osteoblast cell attachment and proliferation. This approach involves the surface functionalization of titanium implants with primary amine groups (–NH₂) used as anchor points to attach AMPs (KR-12) covalently via stimuli-responsive linking arms. These linking arms directly affect the AMPs conformation and their antimicrobial activity; also help the AMPs act specifically on bacteria but not osteoblast cells.

RESULTS: Analytic methods such as X-ray photoelectron spectroscopy (XPS), Fourier-transform Infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Confocal Raman Spectroscopy (CRS) are used to confirm the successful covalent attachment of the AMPs on the surfaces. The XPS and FTIR results reveal that the optimum percentage of the primary amine groups on the surface is 22 % where AMPs can be attached with highest efficiency without showing any toxicity. The CRS and AFM results prove the uniformity of the AMPs immobilized on the surface. Besides, the in vitro antibacterial activity of the AMP-immobilized implant evaluates its efficiency against *Staphylococcus aureus* and *Escherichia coli* as implant-related infections. Moreover, Biological tests assess the cell compatibility, adhesion, and proliferation on the proposed antimicrobial coating.

DISCUSSION & CONCLUSIONS: These peptide-immobilized based coating, involving stimuli-responsive polymers as linking arms, can be fabricated easily and cost effectively, which provide an opportunity toward bacterial infection prevention for a variety of orthopedic implants with positive impact on cells adhesion and growth.

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Keywords: Bone and bone disorders (osteoporosis etc), Polymers - natural / synthetic / responsive

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Silver Nanoparticle Generation to address Implant Infection Rates in Auricular Implants.

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INTRODUCTION: Infection remains a major problem in the current gold standard of auricular implants as *S. aureus* and *S. epidermidis* are frequently observed causing infection at implant sites leading to the implant requiring further treatment using antibiotics or ultimately, the implant being removed from the patient altogether.

Silver nanoparticles (AgNPs) offer a potential means of overcoming implant infection whilst avoiding adding to the emergence of many antibiotic resistant strains of bacteria. Silver has been observed to induce a high degree of structural and morphological changes to the bacterial cells leading to cell death and as such, silver nanoparticles are able to induce bacterial death through two methods, elution of silver ions alongside physical contact with bacterial cells leading to cell death. PCL discs were impregnated with silver nanoparticles using two methods, acid ice bath and dopamine soaking, with the presence of AgNPs confirmed in SEM/EDS. AgNP discs were then cultured with L929 mouse fibroblast cells to assess the cytotoxicity with preliminary results showing low toxicity in the presence of low to medium concentrations of AgNPs. Bacterial strains are currently being exposed to silver impregnated PCL discs to assess their antibacterial activity.

METHODS: AgNP generation was achieved through acidic ice baths which utilises Silver Nitrate, Sodium Borohydride and Mercaptosuccinic Acid in a in-situ reduction reaction. AgNP generation was also carried out using a simple one-step synthesis method using Dopamine Hydrochloride and Silver Nitrate.

Cell viability was assessed through the culture of L929 cells on the surface of AgNP/PCL discs using ReadyProbes Cell Viability kits.

Antibacterial activity was assessed by culturing *S. aureus*, *S. epidermis* and *E. coli* K12 alongside the AgNP/PCL discs and mapping the resulting exclusion zones.

RESULTS: Preliminary results indicate low levels of cytotoxicity following L929 exposure to AgNP discs, despite the generation technique used. At higher concentrations the Dopamine Soak method appears to yield higher levels of cell death in comparison to that observed in the acid ice bath method. Antibacterial activity assessments are still ongoing.

DISCUSSION & CONCLUSIONS: AgNP generation data shows strong formation of AgNPs when both generation techniques are used but with varying levels of uniformity and distribution between the AgNPs with the varying concentrations of reagents used, further work is planned to assess whether exposure to heat during AgNP generation will address this. At higher concentrations near total cell death is observed and a balance must be determined between antibacterial activity and cytotoxicity to ensure the best patient outcome.

Keywords: Infection, Additive manufacturing

PP-223

Modelling in vitro three-dimensional tendon wound healing with equine adult, foetal and embryonic stem cell-derived tenocytes

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INTRODUCTION: Tendon injuries are common in equine and human athletes. Tissue healing occurs via the formation of scar tissue, resulting in high re-injury rates. However, foetal tendons heal scarlessly, possibly attributed to differences in cell migration following injury. Previous in vitro studies demonstrated that foetal and adult tenocytes differ in their global gene expression profiles when cultured in a three-dimensional (3D) collagen gel, with an overrepresentation of genes involved in cell migration. Furthermore, the transcriptomic profiles of embryonic stem cell (ESC)-derived tenocytes resemble foetal, not adult tenocytes, suggesting ESC-tenocytes could be utilised to study the impact of cell migration on tendon wound 'healing' in vitro.

METHODS: In 2D, tenocytes from adult, foetal, and ESC-derived sources were cultured in 2% FBS and the presence or absence of up to fifteen factors (three concentrations and vehicle) that have been demonstrated to be upregulated following a tendon injury. Proliferation was assessed after 72 hrs and cell migration was assessed every 10 minutes for 72 hrs using standardised scratch assays. The five factors which provided the greatest effect were tested in tenocyte co-culture migration assays for the three pairwise comparisons utilising GFP-labelled adult and foetal cells. In 3D culture, tendon constructs were 'injured' for 30 min to 7 days to establish the rate of tenocyte-induced 'healing' of the tendon constructs for each cell type. This wound model will be used to assess the rate of construct 'healing' in the presence of different factors. Finally, those factors which produced the greatest impact in the 2D co-culture experiments will be used to stimulate co-cultured 3D tendon constructs (adult and foetal, adult and ESC-tenocyte).

RESULTS: Preliminary data shows that several factors differentially influenced proliferation and migration across the cell types in 2D culture. The use of ibidi chambers allowed the co-culture of GFP-labelled and non-labelled cells to enable direct comparison of migration by the different cell types. For the 3D culture experiments, we successfully established a wound model, where artificial tendon 'wounds' administered for three days 'heal' by day 10-12 for all tenocyte cell types.

DISCUSSION & CONCLUSIONS: These initial findings suggest that equine tenocytes isolated at different developmental stages respond differently to various factors upregulated after a tendon injury which may account for differences in healing outcomes between adult and foetal cells. However, work is ongoing to establish if ESC-tenocytes behave similarly to adult or foetal cells.

ACKNOWLEDGEMENTS: Petplan

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Stem cells - general

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Electrical stimulation for wound healing - More than skin deep

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INTRODUCTION: An electric field is actively maintained across the epidermis, and, upon injury, electric cues guide wound healing. To treat chronic wounds, electrical stimulation (ES), has shown promising results, but with varying efficacy due to limited understanding of its principle of action. Current ES devices mainly target keratinocyte migration, insufficient in treating full thickness wounds, where the dermis must first be rebuilt. Despite lack of information on the electric field governing the dermis, conceptual models only regard it as a passive component. Here, we hypothesise that the connection with the rest of the body enables this layer to act as a grounding connection. We report the effects of this hypothesis on the dermal layer and its cellular residents surrounding a wound, with and without ES.

METHODS: An in-silico FEM model of a skin segment was designed, meshed, and simulations were performed using the COMSOL Multiphysics 5.2 Electric Currents module. The model adapts the geometry from1, to include infinite boundary elements. Electric potential boundary conditions on the top and bottom surface of the epidermis are used to model the transepidermal voltage of 30 mV. Electroactive wound dressings are modelled by controlling the voltage distribution on the stratum corneum top surface. Our hypothesis is modelled by grounding the bottom surface of the dermal infinite boundary elements.

RESULTS: We report simulation results for the electric field and current density within intact skin, wounded skin with and without exogenous electrical stimulation, comparing the current dogma with the hypothesis of an electrically active dermis.

DISCUSSION & CONCLUSIONS: Our model correctly predicts the intact skin transepidermal electric field of around 100 V/m, the wound edge electric field of around 200 V/m. While an electrically active dermis has no significant effects on intact skin, upon wounding, instead of all current being drawn to the surface, part of it is drawn into the intact dermis. This potential endogenous electrotaxis cue for dermal cells is reversed by conventional ES with a cathode covering the wound. Consequently, macrophage and fibroblast migration to the wound bed may be inhibited. Validation of this hypothesis is ongoing.

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Keywords: Wound healing, In silico models

PP-225

Bacteriophages and Mesenchymal Stem Cells/Aggregates in/on Gelatine Microspheres for Infected Wound Healing

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INTRODUCTION: This study attempts to produce bifunctional gelatine microgels/spheres carrying bacteriophages as antibacterial agents and mesenchymal stem cells (MSCs) for regeneration of both soft and hard tissue as potential hybrid materials for combinational therapy.

METHODS: Both plain gelatine and its cationized forms were used to prepare spherical microgels by dispersing gelatine solution within an oil phase to obtain water-in-oil dispersions that resulted spherical beads which were then cross-linked de-hydrothermally. As a model target *Escherichia coli* and its specific bacteriophages were propagated in standard bacterial cultures. Bacteriophages were labelled by fluorescence dyes for following of both loading and release on/from the gelatine microgels. Nanoemulsions carrying different amounts of bacteriophages were loaded by incubation dry gels with nanoemulsions in proper volumes - sucking the liquid phase by the dry gels.

MSCs were isolated from the adipose tissue from mice, cultured and aggregates were formed with or without the microgels both plain and the ones carrying bacteriophages. The aggregate forms/sizes, cell densities and viabilities were followed in two week's 3D cultures.

RESULTS: Gels with different cross-linking densities swollen in the aqueous phase at different extents were produced in the size range of 50-100 micron. Bacteriophage loadings within the microgels were almost 100%. The release of bacteriophages (both rate and amount) were different depending on the initial loading, properties of the beads (including size, cross-linked density, type of gelatine used) and environmental conditions (including temperature, pH, existence of specific digesting enzymes).

Note that gelatine gels were used as scaffolds for the cells/aggregates. The aggregate of the MSCs were in spherical forms with high cell densities and viabilities which were depended on the microgel formulation and 3D culture conditions.

DISCUSSION & CONCLUSIONS: The gelatine microgels were swollen in the aqueous phase at different extents mainly depending on cross-linking densities. The ratio of bacteriophage nanoemulsion volume and the amount of dry gelatine beads were adjusted to reach very high phage loading densities. Phage loading densities, gelatine bead properties and environmental conditions changed the release behaviour as expected. Successful MSCs aggregation around the gelatine spherical beads allowed us to prepare bioactive hybrids for tissue regeneration. These biofunctional hybrid materials - gelatine microgel beads containing bacteriophages and MSCs aggregates could be used especially for healing of infected wounds (both soft and hard tissue) which is under-investigation in vivo models.

Keywords: Wound healing, Infection

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Platelet-rich fibrin prevents the toxicity of Pamidronate on oral fibroblasts

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INTRODUCTION: Medication-related osteonecrosis of the jaws (MRONJ) is an often-severe complication presented with non-healing oral mucosal wounds and necrotic bone exposure¹. The disease is commonly found in patients under bisphosphonate therapy, such as Pamidronate (PA), for the treatment of osteoporosis and bone malignancies². With no definitive treatment, the management of MRONJ is still challenging³. The use of platelet-rich fibrin (PRF) as an additive treatment for MRONJ patients has been studied as PRF has demonstrated a therapeutic role in oral tissue regeneration⁴. However, it is unclear how PRF enhances the healing of MRONJ wounds. Thus, we aimed to evaluate the effect of PRF on cellular behavior of PA-induced toxicity of oral fibroblasts *in vitro*.

METHODS: Oral fibroblasts isolated from buccal biopsies were used in this study. PRF was obtained from blood samples of healthy donors. Conditioned medium (100%) containing secreted growth factors and proteins from PRF was prepared by incubating PRF with cell culture medium for 3 days. Oral fibroblasts were cultured with PA in combination with three different concentrations of PRF-derived conditioned medium (10, 20, and 50%). Cell processes involved in the wound healing process were investigated to determine the bioactivity of PRF. The metabolic activity, migration, and proliferation were assessed using an MTT assay, cell exclusion zone assay, and flow cytometry, respectively.

RESULTS: PA (100 μ M) was shown to reduce the metabolic activity and proliferation of oral fibroblasts after 72 hours. All concentrations of PRF-derived conditioned medium markedly increased the metabolic activity but had no effect on cell proliferation. PA at a sub-toxic concentration (30 μ M) negatively affected the migration rate of fibroblasts. Adding 50% PRF-derived conditioned medium significantly improved the migration of PA-treated cells compared to the treatment with PA alone.

DISCUSSION & CONCLUSIONS: These data demonstrate the positive effect of PRF on oral fibroblasts by protecting cell metabolism and migration from Pamidronate toxicity. This study highlights the potential role of PRF on promoting the healing of MRONJ wounds. The investigation on 3D tissue-engineered oral mucosa is currently being examined to confirm the biological effect of PRF.

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Keywords: Wound healing, Biologics and growth factors

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Antimicrobial peptide-modified bacterial cellulose-silver nanoparticles composite wound dressing for treatment of wound infections

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INTRODUCTION: The skin is the largest organ of the human body. Wounds disrupt the normal function of the skin. Wound infections can delay wound healing and lead to complications resulting in wound chronification. Bacterial cellulose (BC) is an effective wound dressing material but lacks antimicrobial properties and bacteria in the wounds can thrive in the protected environment under the dressing. Strategies to introduce antimicrobial substances in BC, such as silver nanoparticles (AgNPs), and antimicrobial peptides (AMPs) have been extensively explored, but typically involve strategies that requires harsh chemical treatments that are complex, time consuming and may have negative impact on the properties of BC.

METHODS: We have developed a method based on colloidal self-assembly to create AgNP-BC composite materials. Synthesized AgNPs of varying concentrations (30 nM, 15 nM, 7 nM) were allowed to adsorb to BC under shaking at defined ionic strength to promote close contact between the nanoparticles and the fibrils. Since the use of AgNPs can be problematic from both a biocompatibility and environmental aspect, we adsorbed a de novo designed AMP on the nanoparticles to be able to reduce the amount AgNPs.

RESULTS: We show that the BC-AgNPs can be obtained by means of self-assembly using pre-synthesized AgNPs. The amount of AgNPs could be easily tuned using different concentrations of AgNPs. The BC-AgNP dressings showed a pronounced zone of inhibition (4.5 mm) of *Staphylococcus aureus* on agar plates. Further functionalization with AMPs resulted in wound dressings with significant antibacterial and bactericidal activity, even for the lowest concentrations of AgNPs used.

DISCUSSION & CONCLUSIONS: The proposed strategy simplifies synthesis of AgNP-functionalized BC and provides an affordable antimicrobial wound dressing. The possibilities to further functionalize the AgNPs with AMPs minimizes the need to use excessive concentrations of AgNPs, which decreases cytotoxicity while retaining high antimicrobial activity.

Keywords: Biomaterials, Skin

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Magnesium Hydroxide Nanoparticles Modified collagen/silk fibroin composite scaffold for infected wound healing

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INTRODUCTION: Infected wound induced by pathogenic bacteria have caused considerable threats to global health. Antibiotics have been widely used as the traditional treatments for infected wound, while the improper utilization has led to multidrug-resistant bacteria. Wound dressings constructed with sponge porous scaffolds have been applied to managing infected wound. Natural polymers represented by collagen (Col) and silk fibroin (SF) have been widely used in constructing wound dressings due to their wide sources, strong mechanical properties and excellent biocompatibility. However, weak angiogenesis ability and poor antibacterial properties hinder their further applications. Magnesium hydroxide nanoparticles ($Mg(OH)_2$) have been considered ecofriendly nanomaterials with excellent antibacterial activity, low toxicity, and good biocompatibility. Therefore, we infer that it is possible to construct $Mg(OH)_2$ -Col-SF porous scaffolds to promote the repair of infected wounds.

METHODS: In this study, bioactive natural polymers such as collagen and silk fibroin were used as the main raw materials, supplemented by $Mg(OH)_2$ nanoparticles to enhance antibacterial properties. $Mg(OH)_2$ was prepared by one-pot mixing, freeze-drying, and cross-linking methods. The fabricated $Mg(OH)_2$ -Col-SF porous scaffolds were characterized by physicochemical properties, biocompatibility and antibacterial properties in vitro. Then the rat infected wound model was constructed, and the healing effects were evaluated by gross photo and histological staining.

RESULTS: The fabricated $Mg(OH)_2$ -Col-SF scaffold has a loose and porous structure under SEM. Good mechanical properties, hydrophilicity, and water absorption were observed. Furthermore, good antibacterial effects towards *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were observed. Cell experiments showed the $Mg(OH)_2$ -Col-SF scaffold could promote proliferation of fibroblasts. In addition, $Mg(OH)_2$ -Col-SF scaffold exhibited good antibacterial activity, low toxicity, and accelerated wound repair effects in vivo.

DISCUSSION & CONCLUSIONS: The $Mg(OH)_2$ -Col-SF scaffold was successfully prepared in this study, which has good mechanical properties, antibacterial, and can accelerate the repair of infected wounds. The possible mechanism lie on the promoted angiogenesis, anti-inflammatory, and accelerated collagen deposition.

Keywords: Wound healing, Skin

PP-229

Enhanced Antimicrobial Properties of Scaffolds for Wound Healing Applications

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INTRODUCTION: Tissue engineering is the use of a combination of cells, engineering methods and suitable biochemical and physicochemical factors to improve or replace the biological tissues. Tissue engineering involves the use of a scaffold for the formation of new viable tissues for medical applications. Polymeric scaffolds have been used in tissue engineering applications because of their advantages such as biocompatibility and bioavailability. Boron has an important role in several metabolic pathways. This project designs a new fabrication protocol for boron and Hypericum perforatum oil containing scaffolds and assess their synergistically effect on characteristic/antimicrobial properties of scaffolds.

METHODS: Polyvinyl alcohol (PVA) with molecular weight (Mw) of 85000-124000 and chitosan with low molecular were weight used to produce scaffolds by solvent casting method. H. perforatum oil was used to produce scaffolds at different combinations (0.25, 0.5 % vol/vol). Boron source, sodium pentaborate pentahydrate (NaB, 30 and 35 % w/w, weight of total polymer amount) was also added to the process. The hydrophobicity of scaffolds was also characterized by water contact angle experiment. Mechanical properties of the scaffolds were analysed by tensile machine. The cytotoxicity of scaffolds was analysed with MEF cells. Surface antimicrobial activity of membranes were determined by modifying shake flask method. A gram positive (*S. aureus*), a gram negative (*E. coli*) bacteria and a yeast (*C. albicans*) species was used for antimicrobial tests.

RESULTS: The contact angle results of all samples are in between $86.29^\circ \pm 3$ and $101.25^\circ \pm 2$ and it can be seen that, adding H. perforatum oil increased the contact angle slightly. Mechanical tests showed that with different oil and NaB concentrations, the elongation at break of the membranes changes slightly. In addition, according to the antimicrobial activity results antimicrobial activity of membranes increased with increasing NaB concentration. H. perforatum oil addition also has a positive effect on antimicrobial activity.

DISCUSSION & CONCLUSIONS: A novel potential therapeutic solution for wound healing applications, which combines the PVA/chitosan polymer and H. perforatum oil/NaB has been developed. It can be concluded that, all scaffolds had good cytocompatibility. NaB and H. perforatum oil concentrations affected the characteristic properties and antimicrobial response of the scaffolds.

Keywords: Wound healing, Skin

PP-230

Melt-electrowritten fiber-reinforced hydrogels for sustained release of human induced pluripotent stem cell-derived extracellular vesicles towards cell-free neovascularization

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INTRODUCTION: Extracellular vesicle (EV)-based approaches have shown promise for the treatment of myocardial infarction- and ischemia-associated heart failure. In particular, EV derived from cardiac progenitor and human induced pluripotent stem cells (hiPSC) have demonstrated pro-angiogenic activity [1]. However, translational challenges persist for developing suitable delivery vehicles that prolong EV exposure to target organs. Here, melt-electrowritten (MEW) fiber-reinforced gelatin methacryloyl (GelMA) hydrogels were examined as efficient, tunable platforms for sustained delivery of human induced pluripotent stem cell (hiPSC)-derived EV.

METHODS: hiPSC were expanded in serum-free conditions. EV were isolated from conditioned culture medium by density ultracentrifugation, probed for specific EV markers, and characterized by nanoparticle tracking analysis and transmission electron microscopy. hiPSC-EV were labelled with lipophilic dye PKH26 and incorporated into 3, 6, and 12% GelMA+LAP hydrogels, followed by UV crosslinking. Hydrogels were cast on molds over well-organized polycaprolactone fiber meshes (hexagonal pattern) fabricated by MEW. EV stability and release from pristine and fiber-reinforced hydrogels were measured over 14 days. Stability of EV cargo released from hydrogels over 14 days, in particular of endothelial cell migration-associated miRNA, was assessed by RT-qPCR. Stiffness and stress relaxation of pristine and fiber-reinforced hydrogels were evaluated by compression testing. EV uptake and tube formation was assessed in human umbilical vein endothelial cells (HUVEC).

RESULTS: Pristine GelMA hydrogels showed lower EV release rates at higher hydrogel concentrations, with 12% GelMA providing sustained EV release over a period of 14 days. Fiber mesh incorporation was observed to a) increase compression moduli and delayed stress relaxation of GelMA hydrogels, resulting in up to a 4-fold increase in relaxation time, and b) delay EV release as a result of altered hydrogel relaxation and stiffness, compared to pristine GelMA. Mathematical modelling of EV release curves showed Fickian diffusion to be the predominant release mechanism. During the 14-day release period, the EV retained their ability to be internalized by HUVEC.

DISCUSSION & CONCLUSIONS: Our data demonstrate that GelMA hydrogels enable the sustained release of hiPSC-EV over time, which can be further tailored by adjusting hydrogel formulation as well as introducing fiber mesh reinforcements. Overall, this tunable, sustained release could contribute to enhancing EV retention and optimize the release period in order to improve

therapeutic efficacy upon in vivo local delivery.

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Keywords: Hydrogels and injectable systems, Induced pluripotent stem cells

PP-231

Enhanced and localized pressure-mediated non-viral gene delivery

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INTRODUCTION: Topically-applied therapies must not only be effective at the molecular level but also efficiently access the target site which can be on milli/centimetre-scales. This bottle-neck is particularly inhibitory for peptide and nucleic acid macromolecule drug delivery strategies, especially when aiming to target wounded, infected and poorly perfused tissues of significant volume and geometry. Methods to drive fluid-flow or to enhance physical distribution of such therapies after local administration in such accessible tissues (skin, eye, intestine) would be transformative in realizing the potential of these therapeutics. We previously developed a technology termed GAG-binding enhanced transduction (GET) to efficiently deliver a variety of cargoes intracellularly, using GAG-binding peptides and cell penetrating peptides (CPPs) in the form of nanoparticles (NPs).

METHODS: Herein, we demonstrate that most simple GET transfection formulation is relatively poor in diffusing into tissue (collagen scaffolds). Changing NP physicochemical properties can enhance penetration, however the use of a pressure differential, generating fluid-flow significantly enhances effective gene delivery through milli/centimetre scales. We adapted clinically used pressure systems to administer both negative (Negative pressure (NP) wound therapy; NPWT) and positive pressures (PP; Insufflator).

RESULTS: Pressure-differences generated enhanced distribution and we were able to show for the first-time localized gene transfer in vitro in cell scaffolds.

DISCUSSION & CONCLUSIONS: The ability to simply control intra-tissue localization of gene delivery on millimetre scales using pressure application will facilitate new drug delivery strategies for accessible tissues. Importantly site-specific enhancement of penetration and activity of novel nanotechnologies and gene therapeutics could be transformative for future regenerative medicine strategies.

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Keywords: Gene therapy, Biomechanics / biophysical stimuli and mechanotransduction

PP-232

Next Generation Phage Display for Brain Cancer Specific Non-Viral Gene Delivery

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INTRODUCTION: Glioblastoma multiforme (GBM), the most prevalent primary malignant brain tumour in adults, has a 5-year survival of 7.2%¹. Standard treatment consists of maximal surgical resection, chemotherapy using temozolomide (TMZ), and radiotherapy². Gene therapy could offer new treatments but requires delivery vehicles to facilitate uptake and protection of genetic cargo. The Glycosaminoglycan (GAG)-binding Enhanced Transduction (GET)-peptide electrostatically forms nanoparticles with nucleic acids and enhances transfection through a synergistic effect between a GAG-binding domain and cell penetrating peptide (CPP)³. Next Generation Phage Display (NGPD) and sequencing (NGS) could facilitate screening of GET-variants for identification of GBM-targeting GET-peptides. Previous bio-panning of phage libraries for uptake into KNS42 (paediatric GBM cell line) resulted in identification of potential targeting peptides KNS3RL and KNS3LR.

METHODS: Agarose gel electrophoresis and YO-PRO-1 assay were applied to show complexation with DNA. To investigate transfection, samples were analysed for GLuc expression as described by the BioLux GLuc Assay kit (New England Biolabs).

RESULTS: YO-PRO-1 results showed KNS3RL, KNS3LR, and control peptide (FLR) complexed with DNA by charge ratio (+/-) 3 as fluorescence decreased to 1.6, 0.9, and 1.2% respectively. Agarose gel electrophoresis results showed by charge ratio 2, no DNA had run into the gel. Luciferase results showed KNS3RL was specific for KNS42 cells with a fold difference transfection of ~128.

DISCUSSION & CONCLUSIONS: YO-PRO-1 is a carbocyanine dye that fluoresces when bound to dsDNA. Thus, reduction in fluorescence indicates peptides are removing the dye through complexation with DNA⁴. This was supported by the lack of free DNA seen after charge ratio 2 in the agarose gels suggesting it is within complexes with the peptide. The luciferase results show low KNS42 transfection. However, when investigating specificity it is equally important to reduce off target effects. As such KNS3RL seems promising as it transfects a difficult cell line KNS42 ~128 fold more than the easy to transfect NIH3T3 cell line. These results show that phage isolated peptides can complex DNA and specifically target GBM cells. Future work could be done in generating a library which screens for transfection rather than just uptake of GBM cells.

ACKNOWLEDGEMENTS: Acknowledging Brain Tumour Charity support for a New Ideas Award to J. Dixon to isolate the KNS3 peptide. The BBSRC for PhD sponsorship.

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Keywords: Gene therapy, Cancer

PP-233

Development of Regulated Gene Vector to Restore MeCP2 Activity to Treat Rett Syndrome

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INTRODUCTION: Methyl CpG binding protein 2 (MeCP2) is a nuclear protein that regulates the expression of a wide variety of proteins in neuronal cells. The deficiency of X-linked MeCP2 causes Rett syndrome (RTT), a neurological disorder with severe mental disability that affects mostly females. An increase in MeCP2 protein, by genetic duplication, causes another disorder known as MECP2-duplication syndrome, with similar phenotypes to RTT. Therefore, any therapy replacing MECP2 activity must be very tightly regulated and achieve endogenous levels of activity. Our aim is to develop a regulated gene vector to express MeCP2 protein in the therapeutic range. We are exploiting RNA nuclease CasE to control the amount of MeCP2, with minimal side effects.

METHODS: We used ubiquitously expressed (EF1 α) CasE to regulate the expression of the reporter gene (Gaussian (g)Luc) regulated by CasE through its target site sequence, cloned into its expressed mRNA (CasErec). We generated ubiquitous (EF1 α) and MeCP2-promoter (MeP) driven vectors and EF1 α driving CasE in various orientations to reduce non-neuronal cell transcription, hence decreasing the side effect. The CasE copy number was modulated using upstream open reading frames (1,2,4) which inhibit the effective translation of CasE and therefore its protein amounts and its regulation on the reporter mRNA. The plasmids were delivered using our Glycosaminoglycan-binding enhanced transduction (GET) system developed by our lab (FLR:FLH).

RESULTS: CasE-mediated regulation was effective to reduce the initial transient overexpression of the reporter gene by 90% on the first day. The level of regulation was inversely correlated with the CasE copy number. In HEK 293T cells, the expression of inert-CasE-MeP-CasErec-gLuc in the same orientation was 38.9% higher than the opposite orientation on the third day, showing that orientation of the system is important for overall expression levels.

DISCUSSION & CONCLUSIONS: We have shown that the CasE was effective as transgene regulator in the designed cassette, which opens for the possibility to use it to regulate MeCP2 levels as a gene therapy.

Keywords: Gene therapy, Nervous system (brain-central-peripheral / disorders)

PP-234

Identification and rational design of glial cell line-derived neurotrophic factor (GDNF) peptide mimetics

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INTRODUCTION: Parkinson's disease is the second most prevalent neurodegenerative disorder, affecting over 6.1 million individuals worldwide and with no cure, there is an urgent need for advanced therapeutics. Glial cell-derived neurotrophic factor (GDNF) has shown promise in clinical trials when locally delivered to the brain, however patients required repeated dosing due to the short half-life in vivo. Peptide growth factor mimetics can be used to overcome this, with an increase in stability and a decrease in size. Therefore, the aim was to design a novel GDNF mimetic using rational design and phage display screening methods.

METHODS: PyMOL, SpotOn and Peptiderive applications were utilised to identify key amino acids contributing most to GDNF-GDNF family receptor alpha 1 (GFR α 1) binding, known as hot spots. SpotOn and Peptiderive identified hot spots by performing theoretical alanine mutagenesis studies on GDNF to calculate the change in ΔG binding. In addition, commercial phage-displayed 7-12-mer linear and cyclic peptide libraries were screened against GFR α 1 to identify tightly binding sequences.

RESULTS: From inspection of the GDNF-GFR α 1 crystal structure and the computational alanine mutagenesis studies, two GDNF hot spot sequences were identified. Hits from phage display screening are currently being sequenced.

DISCUSSION & CONCLUSIONS: Two hot spot regions were identified within GDNF which are essential for the growth factor to bind to the GFR α 1 co-receptor. The hot spots identified in the computational and phage display screening will be used to rationally design GDNF mimetics, based on frequently occurring moieties. Mimetics will then be synthesised using solid-phase peptide synthesis and tested in GFR α 1 binding assays.

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Keywords: Biologics and growth factors, Nervous system (brain-central-peripheral / disorders)

PP-235

Co-cultivation of bone progenitor cells to analyze the effect of BMP-2 on bone remodeling processes

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INTRODUCTION: Bone tissue appears inert but is highly dynamic, with bone formation by osteoblasts and bone resorption by osteoclasts. Several local and systemic factors are involved in this process, for example, bone morphogenetic protein 2 (BMP-2). BMP-2 has been shown to promote osteogenic differentiation. Thus, there is great interest in the clinical application of BMP-2 in treating critical-size defects. To investigate the effect of BMP 2 in bone remodeling, an indirect co-culture of human primary pre-osteoblasts (OBs), mesenchymal stem cells (MSCs), and peripheral blood mononuclear cells (PBMCs) was used.

METHODS: For indirect co-culture, PBMCs (n=1) were cultured in wells, while OBs (n=3) and MSCs (n=3) were cultured in cell culture inserts. BMP-2 stimulus (1 µg/ml) was provided via the medium in the insert. The cultivation time was seven days, and subsequently, RUNX Family Transcription Factor 2 (RUNX2), Secreted Phosphoprotein 1 (SPP1), Acid Phosphatase 5, Tartrate Resistant (ACP5), and Carbonic Anhydrase 2 (CA2) were examined as marker genes for human osteoblasts and osteoclasts. In addition, the secretion of the proteins OPG and RANKL was analyzed since the OPG-RANKL ratio is an important factor in osteoclast activation.

RESULTS: In the co-culture of OBs and MSCs, a significant increase was present for both RUNX2 (p=0.0008) and SPP1 expression (p=0.015) after BMP-2 exposure. When OBs and MSCs were co-cultured with PBMCs, this effect was no longer evident; instead, co-culturing with PBMCs resulted in significantly lower RUNX2 expression in OBs than in the monoculture of OBs (p= 0.001). Co-cultivation with OBs, MSCs, and BMP-2 treatment resulted in increased transcript levels of ACP5 (p=0.0008) and CA2 in PBMCs. Quantification of RANKL showed a significant increase after BMP-2 exposure for co-culture of OBs and MSCs (p=0.025) and MSCs and PBMCs (p=0.0146). No significant effects were present for OPG. However, the OPG to RANKL ratio was significantly changed towards RANKL.

DISCUSSION & CONCLUSIONS: Co-culturing of human OBs, MSCs, and PBMCs revealed that exposure to BMP-2 not only promotes osteogenic differentiation but also activates bone remodeling processes, as seen by the increased gene expression of ACP5 and CA2 and the change in OPG to RANKL ratio. However, the presence of multiple cell types also indicates that the effects of BMP-2 on osteogenesis are less evident than in monoculture.

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Keywords: Bone and bone disorders (osteoporosis etc), Differentiation

PP-236

Proteomic characterisation of equine platelet rich plasma and their extracellular vesicles

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INTRODUCTION: Platelet-rich plasma (PRP) is applied as therapeutic in tendon injury and osteoarthritis in humans and horses to aid the repair of the extracellular matrix in these age-associated conditions. PRP contains high concentrations of soluble growth factors as well as extracellular vesicles (EVs). EVs are nanoparticles involved in intracellular communication. Despite its wide use in clinical practice, the optimal composition of PRP has not been determined, resulting in variable clinical outcomes.

METHODS: Blood samples (n=3) were collected from horses into acid citrate dextrose tubes. Plasma was isolated following double centrifugation, and PRP was produced using a commercial filtration kit. EVs were isolated from PRP and plasma using differential ultracentrifugation and characterised. Plasma and PRP protein were equalised using Proteominer columns followed by on-bead tryptic digestion. Protein in EV samples was extracted using urea lysis buffer and sonication and subjected to in-solution tryptic digestion. All samples were analysed with liquid chromatography-tandem mass spectrometry and resulting datasets analysed using Ingenuity Pathway Analysis (IPA).

RESULTS: Total number of proteins were identified for each group (PRP -575, Plasma-558, PRP-EVs-209 and plasma- EVs-195). Principal component analysis of all proteins showed separation of plasma and PRP proteins (explained variance in principal component 1 of 54.7%) and EV-protein samples (explained variance in principal component 1 of 93.6%). Fold change analysis (significant = >2- fold change) found PRP to have expression of 395 proteins increased, and 93 decreased compared to plasma. In PRP-EVs 130 proteins demonstrated a significant increase and 98 decrease compared to plasma-EVs. For the PRP and PRP-EV proteome significant pathways were identified using all identified proteins, included protein synthesis ($p=1.57 \times 10^{-5}$ - 1.16×10^{-23}) and organisation of cytoskeleton ($p=2.62 \times 10^{-5}$), respectively.

DISCUSSION & CONCLUSIONS: PRP-EV proteomic cargo was explored in order to find their potential role in the therapeutic mechanisms of PRP. Data is suggestive that EVs should be explored further when considering optimal PRP composition to improve PRP efficacy in clinical practice.

Keywords: Biologics and growth factors, Veterinary

PP-237

Data analysis for stereotype morphology for robust evaluation of MSCs

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INTRODUCTION: In recent year, human cells are new types of medicinal products that are rapidly growing. Human mesenchymal stem cells (MSCs) had started to be approved as both autologous and allogenic cell therapeutic products in the world. Human adipose derived MSCs (hADSCs) has an advantage of feasible access to the origin tissue, and also has the multi-potent for differentiation and immunomodulatory effects [1]. Some reports show that ADSC can provide higher yield in their manufacturing [2]. By such scientific developments, there are growing demands for evaluating the quality of culturing cells non-invasively by images for better quality control in cell manufacturing. Our group has been reporting the morphology-based cell quality evaluation using microscopic imaging system and machine learning. However, in the usage of machine learning in such morphological images, the biggest difficulty was the limitation of trained computer for new data to face the large difference in individual differences. We here report a new morphological data machine learning concept to reduce the experimental volume but enhance the constructed models' performance.

METHODS: Using hADSC lot library with variations of passages, we collected the hADSCs' morphological database from more than 10000 cells covering more than 20000 images, and processed the data to extract the commonly shared "stereotype morphology" by data analysis. By using these stereotyped data of morphology, we tried to construct morphology-based prediction models to predict their decrease in quality.

RESULTS: Our morphological data analysis revealed some preference rules that is especially found in early/late passage samples, and such extracted morphologies enhanced the prediction performance.

DISCUSSION & CONCLUSIONS: Our data showed to use the most of the limited data, by introducing data analysis in multi-parametric morphological parameters for robust machine learning, and it can be applied to the cell manufacturing processes to predict their cellular quality from limited cells.

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Keywords: Stem cells - general, Other

PP-238

Clinical-use Cryopreservation Medium

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INTRODUCTION: Cryopreservation is a common method for long-term biological material storage for later research or clinical use as an important part of advanced therapy medicinal products (ATM). Those clinical applications have to fulfill strict regulatory requirements, e.g. on the chemically defined composition of the cryopreservation medium. As an example, Foetal Bovine Serum (FBS), a common cell-biology R&D component, has to be avoided in any of the steps in the preparation of cell-based medicine, including cryopreservation.

Moreover, none of the components in the preparation of the ATM products can negatively influence the characteristics of the cells. Unfortunately, the most widespread cryopreservation methods are based on the use of cryoprotective agent (CPA) 10% dimethyl sulfoxide (DMSO), which causes several adverse effects, including cytotoxicity, affects cell expression and epigenetics, and affects high proliferation activity and pluripotency to different cell lineages of the human Mesenchymal Stem Cells (hMSC). Accordingly, we employed the High-Molecular Hyaluronic Acid (HMW-HA), the cytoprotective component of the extracellular hMSC's niche, that could provide properties beneficial during stem cell cryopreservation.

METHODS: Cells were cryopreserved with cryomedium in – 80°C freezer. After storage, cryovials were rapidly thawed in a 37° C water bath and subsequently cultivated for 2 weeks to characterize the cell count, viability, and surface marker phenotype (CD73, CD90, and CD49f) by cell counter and flow cytometry.

RESULTS: In our previous research, we identified the cryoprotective ability of the HMW-HA to mitigate DMSO adverse effects during cryopreservation. Partial substitution of potentially harmful DMSO by HMW-HA led to a positive effect on cell survival and proliferation after cryopreservation. Comparison of surface stem cell phenotype markers before and after cryopreservation showed a positive effect on hMSC's stemness marker CD49f co-cryopreserved with HMW-HA in comparison to its controls, while expression of other stem cell markers remained unchanged (Pilbauerova et al., 2022). Based on these findings we prepared the cryopreservation medium on the pharmaceutical grade level, fulfilling all requirements for ATM products.

DISCUSSION & CONCLUSIONS: HMW-HA content facilitates a substantial decrease of potential harmful DMSO, the cryopreservation medium has a defined composition, is animal component free (serum-free), and is sterile with a low level of endotoxin contamination (under 0.05 EU/mg). In general, our cryopreservation medium is easy to use, universal for cells of various origin, and allows long-term cryostorage.

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Keywords: Stem cells - general, Advanced therapy medicinal products

PP-239

Development of Cell Therapies For Liver Disease: An Experimental Flow Model To Optimise Cell Delivery

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INTRODUCTION: Cell therapy has emerged as an alternative treatment to organ transplantation for liver disease. The significance of the therapeutic benefits is hindered by the lack of engraftment to the target tissue. Hence, there is a need for more translational preclinical research to optimise cell therapy protocols. Our research aims to investigate new approaches that could improve the delivery and engraftment of therapeutic cells in the treatment of liver disease. Our study involves the design of an *in vitro* model coupled to a novel mathematical model that together yield mechanistic insights into the role of fluid shear on cell function and can be used as a predictive tool for defining optimal cell delivery conditions. We will validate our *in vivo* using mouse models of liver disease.

METHODS: Mouse hepatic progenitor cells, mouse hepatocytes and human primary liver progenitor cells derived from organoid cultures were circulated on the designed *in vitro* flow model at a range of flow rates for different duration. The effects of these flow rates were assessed on cell viability and the integrin family gene expression. Additionally, these changes were correlated with the ability of the cells to attach to a range of ECM molecules using an ECM cell adhesion array. The theoretical model is based on a systematic reduction of the Navier-Stokes equations, which exploit the small aspect ratio of the vessels and low reduced Reynolds number. Cells are assumed to advect with the flow, and the resulting shear stress they experience is quantified.

RESULTS: In our experimental model cell viability can be maintained at a circulation of 1,59mL/min across all cell types. Expression of key matrix attachment proteins, integrins, is also upregulated providing evidence that cell preconditioning could promote cell engraftment. However, the upregulated integrins varied with cell type and with time after flow. The upregulation of integrins is correlated with the shear stress predictions of the *in silico* model.

DISCUSSION & CONCLUSIONS: Our integrated *in silico-in vitro-in vivo* preclinical framework will guide candidate regimes for cell delivery. This multi-disciplinary project enables optimisation of cell therapy protocols to ensure safe delivery of cells to the injury site and their successful engraftment, facilitating successful clinical translation of next-generation cell therapies for liver disease.

ACKNOWLEDGEMENTS: MRC-UKRMP, MICA: Exploiting *in silico* modelling to address the translational bottleneck in regenerative medicine safety, MR/T015489/1.

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Keywords: Cell therapy,

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Novel Bioactive Calcium Phosphate based endodontic cements with added Hydroxyapatite nanoparticles and antibacterial agent ϵ -Polylysine

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INTRODUCTION: Vital Pulp therapy and Revascularization are endodontic therapeutic techniques that rely on the regenerative capacity of human dental pulp stem cells (hDPSCs). An Ideal material for these techniques should provide antibacterial action, encourage cell proliferation and release essential ions for remineralisation. Other requirements include suitable handling, setting and mechanical properties. Unfortunately, current commercial materials, fall short of fulfilling some of these requirements. Calcium phosphate compounds have inherent biocompatibility, bio-interactivity and bioactivity (apatite-forming capacity). Nevertheless, their application in Endodontics have not been fully explored.

Aim is to improve the bioactivity of a Brushite forming novel calcium phosphate cement, by addition of Hydroxyapatite (HA) nanoparticles in the presence of ϵ -polylysine (PLS) as an antibacterial agent and comparing it to Mineral Trioxide Aggregate (MTA), Biodentine and Dycal.

METHODS: Brushite cement was prepared using equimolar Mono and β -Tricalcium phosphate (TCP) with 800mM citric acid at a powder to liquid ratio of 4:1. This was then modified through TCP substitution by HA (25 or 50 wt%). Additionally, PLS (20 or 40 wt%) was added to the citric acid solution. Setting and dissolution kinetics were determined using FTIR and gravimetrically (n=3). Flexural strength after setting for 24 hours was determined (n=6). hDPSCs viability and adherence on disc surfaces was assessed with SEM and Live /dead (L/D) stain (n=3). Cellular proliferation rate in the presence of different extract dilutions for up to 7 days was assessed using MTT assay (n=6).

RESULTS: Combined FTIR and mechanical studies, showed Brushite formulations set within 30 minutes. Conversely MTA and Biodentine had much slower and different setting chemistries. Flexural strength for the Brushite formulations decreased upon PLS addition (25 ± 2 to 12.14 ± 1 MPa). Strengths of MTA (6.4 ± 0.5 MPa), Biodentine (13.4 ± 1 MPa) and Dycal (3.4 ± 0.5 MPa) were significantly lower ($p < 0.05$). Increase in PLS concentration, accelerated the early Brushite dissolution (first 30 minutes), but this was reduced 50% by HA addition. Commercial cements initially increased in mass before dissolution. L/D assay showed a high ratio of live cells in the HA added groups, this ratio decreased clearly in the HA free formulations, with fewer live cells on the MTA and Biodentine, and none on the Dycal. MTT assay showed cells exposed to extracts from formulations with HA had a higher proliferation rate than control and commercial formulations.

DISCUSSION & CONCLUSIONS: Brushite forming cements with antibacterial agent ϵ polylysine may be a useful candidate as an endodontic cement. Addition of Hydroxyapatite nanoparticles improved the bioactivity.

Keywords: Advanced therapy medicinal products, Biomaterials

PP-241

A combination therapy for the treatment of obesity and obesity-related metabolic disorders

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INTRODUCTION: Obesity is becoming a global epidemic and increases the risk of various metabolic disorders including type 2 diabetes and non-alcoholic fatty liver disease. Although there are several anti-obesity medications approved by U.S. Food and Drug Administration, they have low efficacies in causing substantial weight loss to achieve health benefits and have various side effects. More effective pharmacotherapies are therefore in great need. Metformin is a first line medication for type 2 diabetes, and has been reported to have mild effects on inducing weight loss. Recent data from our laboratory show that recombinant human arginase (rhArg) has potent anti-obesity and insulin sensitizing effects. This study aims to investigate the efficacy of combined treatment of rhArg and metformin on obesity and obesity-associated metabolic disorders.

METHODS: A diet-induced obesity (DIO) mouse model was used for the study. In brief, C57BL/6J male mice were fed a high-fat diet (HFD) for 3 months. By that time, besides exhibiting an increase in bodyweight, the mice had developed hepatic steatosis, elevated blood glucose, impaired glucose tolerance and insulin resistance. The DIO mice were then stratified into 4 groups according to bodyweight, and received single treatment of either metformin or rhArg, or combined treatment of both drugs, or their vehicles. Age-matched mice that were fed a chow diet and treated with vehicles served as the lean control. Glucose tolerance test and insulin tolerance test were conducted during the second month after commencement of the dosing regimen. At the end of 9 weeks of treatment, serum and tissues of various organs were collected for histological, biochemical and molecular analyses.

RESULTS: We found that combined treatment of rhArg and metformin had synergetic effects on inducing weight loss. Moreover, insulin sensitivity and glucose tolerance were significantly improved, and hepatic steatosis was markedly alleviated. We further found that the lipogenesis pathway was greatly inhibited, while lipolysis and lipid oxidation pathways were activated.

DISCUSSION & CONCLUSIONS: In summary, rhArg and metformin show good combination effects on reducing obesity and alleviating various obesity-associated metabolic disorders. Metformin is clinically in use with few adverse effects, and arginine depletion using rhArg has shown to be well-tolerated in clinical trials. Our present findings support that combined treatment of metformin and rhArg has good potential to be developed into a novel therapeutic strategy for obesity and its related metabolic disorders.

ACKNOWLEDGEMENTS: This study is supported by the Lo Ka Chung Charitable Foundation.

Keywords: Disease models,

PP-242

Autologous 3D neural implants for Spinal Cord Injury

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INTRODUCTION: Spinal Cord Injury (SCI) is a devastating medical condition that affects thousands of patients globally, imposing medical, social and economic burden and unfortunately has no effective curative solution.

Tissue engineering-based technologies have the potential to revolutionize the existing approach to treating severely damaged spinal cord tissue. Current developments mostly include the use of synthetic materials as scaffolds and/or allogeneic cells for cell-based therapies that carry a significant risk of triggering an immune response which may lead to rejection and inflammation.

METHODS: We report on a novel tissue engineered product that is a completely autologous neural implant which present the potential to promote tissue regeneration while reducing the risk of implants rejection. T

RESULTS: he implant is composed of a scaffold in the form of a hydrogel, fabricated from the patient's omentum (intra-abdominal fatty tissue), and induced pluripotent stem cells (iPSCs) derived from the patient's blood. The iPSCs are incorporated and differentiated within the hydrogel, forming a mature functioning 3D neural tissue. The implants present typical neural markers, such as neuronal microtubule element b-tubulin, HB-9 gene which is expressed selectively by motor neurons, and vesicular acetylcholine transport (VAcHT) protein as a cholinergic neuron marker.

DISCUSSION & CONCLUSIONS: Feasibility studies in hemi-section chronic mice model demonstrate improved functional results in mice treated with the neural implants emphasizing the potential of the engineered neural implants to regenerate injured spinal cord.

Keywords: Advanced therapy medicinal products, Induced pluripotent stem cells

PP-243

Electrochemical Analysis of In Vitro Electrical Stimulation

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INTRODUCTION: Electrical stimulations (ES) with various intensity, frequency, duration, signal shape, etc. are used to enhance tissue regeneration. However, it is not yet clear which combination of parameters is efficient in each application. A relevant approach to refine these sets of parameters is to reveal the significant electrochemical features linked to the biophysical responses [1,2]. Here, we characterized the electrochemical features of different in vitro ES systems and regimes in biologically relevant conditions.

METHODS: We characterized three different systems: miniaturized devices with electrode plates, coated with either Pt thin film (TF) or Pt nanocolumns (NC) [3]; and an 8-well plate with L-shaped Pt wires (W). Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were measured using a potentiostat. Chronoamperometry was carried out for different ES regimes.

RESULTS: Impedance is significantly reduced when NC are used. The NC effective capacitance is larger than that of TF due to its increased electrochemically active area. The safe limits are between -0.7 V and 1.0 V for both NC and TF. Redox peaks were observed in the same potential ranges for both morphologies. Accumulated charge was considerably larger for both morphologies in the potential regions where oxidation of Pt and adsorption-desorption of H₂ occur, showing its dependency on the applied potential ranges. Moreover, charge per pulse was around two orders of magnitude larger for NC than for TF in every region.

DISCUSSION & CONCLUSIONS: The applied electrical signals are not equal to what cells sense due to the electric double layer at the interface and its characteristics are extractable from EIS.

Chronoamperometry provides the accumulated charge in the system. The sum of charges passed to the extracellular fluid highly depends on electrode material, operation voltage range, and signal shape. Finally, even being in the safe operating voltage range, faradaic reactions [4], responsible for production of reactive oxygen species, change with the amount of energy injected to the system. This energy correlates with specific reaction peaks in the CV curve.

Electrochemical characterization will make possible to compare the energy and charge introduced to the system for different ES protocols.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Nanomaterials (inc graphene)

PP-244

In vitro advanced model to investigate in situ biomechanisms of industrialized electrospun vascular prosthesis

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INTRODUCTION: Recent developments in vascular tissue engineering strategies show a shift towards in situ vascular tissue regeneration based on cell-free synthetic scaffolds promoting tissue regeneration inside the human body. Despite encouraging in vivo proof-of-concept studies, intimal hyperplasia, early stenosis, and aneurysm formation remain prevalent complications in graft implantation. Although they are often attributed to a mismatch between graft properties and haemodynamic induced mechanical loading, resulting in aberrant inflammation and pathological tissue deposition; the underlying biomechanisms remain elusive.

In this context, in vitro and ex vivo models are becoming increasingly more relevant as alternatives to in vivo animal tests, to investigate cells and tissue responses to graft implantation, applying mechano-chemical in vivo-like stimuli. For this purpose, we developed an advanced and versatile culture system to investigate the mutual role of mechanical cues on cells-mediated neotissue formation and the bio-mechanical interaction between graft and native vessel.

METHODS: The developed system allows to perform semi-automated cell seeding and apply in vivo-like controlled stimuli (pre-tensioning, dynamic wall shear stress (WSS) and circumferential deformation). It is composed by a culture chamber, a rotating mixer for semi-automatic cell seeding, two independent fluid dynamic circuits, a pinch-valve to generate a pressure circumferential stress on the sample.

The system was successfully verified by performing bench tests and in silico simulations were done to optimize the seeding and culture protocol. Moreover, we performed first biological tests aimed at obtaining a homogenous cellular monolayer on the graft luminal side. Three-layered electrospun grafts ($\varnothing_{in} = 6$ mm), composed of a nanometric mesh of silk fibroin (SF) and polyurethane (PU) enclosed within SF layers, were manufactured and used for these tests. Human umbilical vein endothelial cells (HUVECs) were seeded and stimulated with a gradually increasing WSS. After that, we evaluated flow-induced HUVECs morphology, cell adhesion to the substrate, cell orientation (SEM, immunofluorescence staining of e.g. F-actin, VE-Cadherin, pFAK, paxillin, ZO-1), and gene expression (e.g. KLF-2, vWF).

RESULTS: We obtained a homogeneously populated graft with endothelial cells oriented along the flow direction. Subsequently, we are going to anastomose the graft to a native vessel harvested by slaughterhouses; in this way, it will be possible to reveal which stimuli differently regulate cells and matrix growth, tissue remodelling and gene expression as early pro-pathological markers.

DISCUSSION & CONCLUSIONS: Through the development of this advanced culture system it will be

possible to establish an accurate in vitro model that allows to investigate the complex biological interactions that occur in vascular tissue engineering.

Keywords: Cardiovascular, Biomechanics / biophysical stimuli and mechanotransduction

PP-245

Development of high-throughput bioengineered platforms for real-time assessment of musculoskeletal rehabilitation

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INTRODUCTION: In vitro 3D bioengineered skeletal muscle is becoming a powerful research tool. Improvements in these models help us to mimic *in vivo* tissue characteristics better than 2D culture. However, when it comes to the data collection, the current design of bioreactors is severely limited by a reliance on destructive endpoint analysis. Therefore, this proposal will consider the design and bioengineering of novel bioreactor systems that enable assessment of skeletal muscle tissue function in real-time, as well as validating the resulting prototype bioreactor systems through comparison of tissue outputs against existing methodologies.

METHODS: C2C12 type I collagen only hydrogels were cultured in a Mantarray™ 24-well plate. The Mantarray™ uses a post-deflection system to assess functionality through contractile force measurements (1). Electrical field stimulation to obtain peak twitch and tetanus data were derived from 3 contractions per tissue sample with standardized separation times of 10 s between contractions. Peak twitch force was determined using a single electric pulse of 3.6 V/mm and 1.2 ms impulse. Maximal tetanic force was measured using a 1s pulse train at 100 Hz and 3.6 V/mm.

RESULTS: A stimulation system prototype, based on tin-plated pins, has been built to be also compatible with the commercial system. After 14 days of culture, 3D musculoskeletal constructs were subjected to functional analysis without the need of a force transducer (current method at the laboratory) and tissues remained intact during the whole process. Tetanus and twitch output were recorded with the Mantarray™ system.

DISCUSSION & CONCLUSIONS: Together, both devices, enable us to stimulate and collect information in real time without end-point measurements. Furthermore, four different connections have been implemented in the device to potentially generate various conditions within the same plate. This would allow a wide variety of conditions and stimulus to be generated with only a single culture set, reducing the number of experiments and the time needed for studying muscle injury, repair, complex diseases, or metabolic models. Eventually, this could facilitate access to an amount of data big enough to work with A.I. models that will help to predict muscle function and behavior based on force output of previously studied tissues.

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Keywords: Bioreactors, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-246

Industrializing 3D organ-on-a-chip: the development of a bioreactor for organoid mass production

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INTRODUCTION: Organoids hold the potential to mimic organ physiology in 3D models. However, organoid production faces critical challenges such as small-scale, high costs and reproducibility. Currently, the scientific literature has been arguing that the convergence of organoids and microfluidic technologies to overcome these limitations. Specifically, microfluidics technologies can reduce labor hours and cost by supporting automated systems and low volume of reagents. In fact, the most crucial challenge faced today by translation of organoids to clinical protocols relies on their cost, mostly based on the high cost of cell culture medium supplies and low-throughput of available materials and protocols. For example, bioprinting protocols need hundreds to thousands of organoids to form tissues and organs in a suitable size for medical transplants. The main objective of this study is to develop a microfluidic bioreactor for organoid mass production.

METHODS: A CAD model was designed containing individualized chambers and channels to accommodate hundreds of organoids. The negative aluminum mold was fabricated using a CNC milling machine. The positive mold (bioreactor prototype) was microfabricated using a pressing system with the thermoplastic Flexdym. Human adipose stem/stromal cells (ASCs) were used to test the capacity of the bioreactor prototype to form spheroids.

RESULTS: Preliminary results showed a homogenous ASC spheroid formation. The viability was tested for up to 2 weeks using live and dead assay.

DISCUSSION & CONCLUSIONS: The bioreactor prototype microfabrication revealed a simple and cost-effective process suitable for large-scale. Induction protocols are in progress to mature ASC spheroid in adipose tissue organoids.

Keywords: Bioreactors, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

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Design and optimisation of a novel fluidised bed bioreactor for the expansion of erythroid progenitor cells

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INTRODUCTION: Worldwide, more than 118 million units of blood are transfused, annually(1). These units are sourced from donors, however whilst demand is rising, donor numbers are falling(2). In the UK alone, ~400 new donors are required daily to maintain provision. Compounding these shortfalls are the risks of infectious blood-borne diseases(1), scarcity of rare blood-group donors, and the extensive blood group typing required to limit undesirable immune responses in patients requiring regular transfusions(3). Safe, reliable, and well-matched sources of blood are therefore required, particularly for its erythrocyte (red blood cell) component. The recent creation of the first erythrocyte precursor cell-line (BEL-A) provides a promising source for the in vitro production of erythrocytes without need of donors(2). However, low yields and high costs pose challenges for their clinical translation. There is, therefore, a need for a novel manufacturing platform in order to facilitate the production of this important therapeutic product.

METHODS: Our research focuses on designing and optimising a novel fluidised bed bioreactor (FBB) to scale up BEL-A cell production to therapeutically-relevant levels. FBBs are an attractive candidate for manufacturing erythrocytes as they can enable superior mass transfer under low-shear compared to traditional systems. Following the successful culture of BEL-A cells within FBBs in our proof-of-concept studies, our current focus is to optimise design and operating conditions to improve yields and bioreactor performance. In addressing this, we have adopted a multidisciplinary approach, combining biological and engineering principles to understand and improve our existing system for suspension cell culture. Key aspects of this include computational fluid dynamics to define flow regimes within the bioreactor, optimisation of fluidisation characteristics through rheometry and media density analysis, and the definition of key biological characteristics underpinning BEL-A cell production scale-up, including cell morphology, spent media analysis, and cellular response to dynamic culture conditions.

RESULTS: Results of this study are a major step towards the efficient manufacture of sufficient precursor cells for maturation into erythrocytes for clinical use.

DISCUSSION & CONCLUSIONS: Furthermore, the FBB, being a platform technology, can be adapted for the manufacture of other similar cell-therapy products.

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Keywords: Enabling technologies, Scaling up & manufacturing

PP-248

Developing equine liver organoids for nutritional studies and (metabolic) disease modeling

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INTRODUCTION: Organoids are 3D cultures that functionally resemble the organ-of-origin. In veterinary medicine most studies describe intestinal and hepatic organoids derived from dogs and cats and, to a lesser degree, horses¹. Culturing organoids in a physiologically relevant setting can reduce the number of animals needed for nutritional, pharmacological, compound, and toxicological screening. Moreover, organoids appear to be excellent models of disease²⁻⁶. The combination of hepatic and intestinal organoids would create a very valuable model to study equine GI-tract disorders and nutritional (patho)physiology, dietary interventions and compound screening. The development and characterization of equine intestinal organoids has been described previously^{7,8}, but not for liver.

METHODS: . Organoid expansion. Freshly sectioned equine livers were minced and enzymatically digested with collagenase II and dispase. Isolated intrahepatic biliary duct fragments were pelleted and cultured in Matrigel domes. Optimization of culture conditions for intrahepatic-derived equine organoids was based on previous publications^{2,3,4}. Most importantly, the optimized equine Expansion Medium (eEM) contains activators of Wnt-signaling. Every 2-3 days, eEM was refreshed. Organoid differentiation. To induce differentiation into hepatic organoids, eEM was tweaked according to a previous publication⁴ and equine Differentiation Medium (eDM) was refreshed every 2-3 days, until harvest (day 7-12).

RESULTS: Organoids derived from intrahepatic bile-ducts expressed typical stem cell and cholangiocyte markers, such as SOX9, CXCL8, and HNF1b. There was no expression of more mature hepatocyte markers such as SLC27A5, FAH, Albumin, and HNF4a. Preliminary data indicate that culture in eDM, in contrast to eEM, resulted in greater expression of FAH, Albumin, and HNF4a.

DISCUSSION & CONCLUSIONS: The establishment of equine liver organoids with either stem/cholangiocyte or hepatic phenotype could provide a model to study equine metabolic disturbances, as was described in lipid-laden feline liver organoids⁹. In combination with equine intestinal organoids, nutritional, pharmacological and toxicological studies should be possible without the need for large numbers of animals in a long-term study set-up.

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Keywords: Disease models, In vivo and animal models

PP-249

Effects of resveratrol and its analogues on the cell cycle of equine mesenchymal stem/stromal cells

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INTRODUCTION: Resveratrol (trans-3,5,4'-trihydroxystilbene) is a polyphenol found in some plants including grape skin and its associated products (1). There are many studies reporting on the beneficial effects of resveratrol on chronic diseases. Resveratrol has a strong effect on the inflammatory phenotype of senescent cells and can reverse cell senescence. Investigations for resveratrol effects on mesenchymal stem/stromal cells (MSC) are increasing in the field of human regenerative medicine (2) because of its strong effect of activating sirtuin 1 (SIRT1) which is associated with biological aging. Although SIRT1 is decreased by long culture of human MSCs (3), RSV promotes cell cycling of MSCs, cell survival and proliferation effects by activating SIRT1 (4). Efficient methods of proliferating and improving the quality of Equine MSC (eMSC) are considered important because eMSC is used as one of the main methods of equine regenerative medicine for musculoskeletal diseases. Studies on the effects of resveratrol and V29, which is one of the novel resveralogues, on bone marrow-derived eMSC (BM-eMSC) are lacking. The objective of this study is to investigate the effects of resveratrol and V29 on the cell cycle of BM-eMSCs in vitro.

METHODS: BM-eMSCs derived from seven horses were evaluated for their cell proliferation capacity using EdU assay and Ki67 antibody following treatment with 5 μ M RSV and 10 μ M V29.

RESULTS: The growth fraction of BM-eMSCs was significantly increased as measured EdU assay by RSV and V29 compared to control cells (control 28.1% $\pm$ 13.8, RSV 31.8% $\pm$ 14.6 and V29 32.0% $\pm$ 10.8 (mean $\pm$ standard deviation). In contrast, the growth fraction as measured by Ki67 antibody was not significantly increased compared to control cells (control 27.0% $\pm$ 11.2, RSV 27.4% $\pm$ 10.8 and V29 27.7% $\pm$ 6.8).

DISCUSSION & CONCLUSIONS: Our results indicated that both of resveratrol and V29 had the effect of promoting the cell cycle of BM-eMSC to the S phase and cell proliferation. Both of resveratrol and V29 would be useful for the expansion of BM-eMSCs for regenerative medicine in clinical use.

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Keywords: Veterinary, Stem cells - general

PP-250

A marine-derived collagen-based skin substitute for skin regeneration: a preliminary study

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INTRODUCTION: Skin wounds are a challenging problem in the medical field and an economical burden. Skin substitutes (SS) are a promising alternative for skin wound healing since they mimic the natural skin structure and support its regeneration. Here, we present preliminary results about the application of an innovative marine-derived collagen-based SS in a secondary intention wound healing model.

METHODS: The collagen used to produce the SS was obtained from sea urchin (*Paracentrotus lividus*) food industry wastes, extracted in its fully native fibrillar and GAG-decorated form [1]. Two wounds (4x4 cm) were surgically induced on the back of three sheep: one of them was treated with the SS and one left untreated (placebo). Clinical evaluations for wound contraction and re-epithelialization were performed every week. At 7, 14, 21 and 42 days, biopsies were collected with a 6 mm² punch in order to perform histopathological (H&E) and molecular (qPCR) analyses. Histological analyses allowed to provide a score to the maturation of granulation tissue (GT) and skin adnexa. Gene expression analysis was performed for Collagen1, Collagen3, VEGF and hair-keratin (hKER).

RESULTS: Clinically, the SS led to a better re-epithelialization than the placebo while no differences in wound contraction were observed. Treated wounds showed a faster histological resolution of GT at 21 days, with no exuberant deposition of scarring tissue at 42 days; this result was further corroborated by the different expression levels of both collagen types at 14 and 21 days.

Concomitantly, the higher expression of VEGF (day 14) was detected. In untreated wounds, on the contrary, GT appeared only at 14 days and did not mature properly leading to dermal fibrosis at 42 days. SS application led to the development of a larger amount and better developed skin adnexa (present since day 14) than untreated wounds: treated wounds started to express hKER since day 14.

DISCUSSION & CONCLUSIONS: At 42 days, a faster re-epithelialization, a better development of skin adnexa and a properly deposition of GT were observed in the SS-treated wounds while the untreated ones showed a hypertrophic epidermis and fibrosis, indicators of a tissue still in the process of healing. Interestingly, VEGF expression might have supported GT maturation. Therefore, our preliminary findings suggest an overall better skin repair of the SS treatment than the placebo.

Future studies should compare these beneficial effects with a commercially available SS (Integra®).

REFERENCES: [1] Ferrario et al. Mar Environ Res. Jul;128:46-57, 2017

Keywords: Biomaterials, Wound healing

PP-251

Platelet lysate does not exert anti-inflammatory effect on monoiodoacetic acid-induced equine persistent synovitis

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INTRODUCTION: In horses, varied tissue repair effects of platelet-rich plasma (PRP) or platelet lysate (PL) have been demonstrated. However, the anti-inflammatory effect of PRP is controversial. To clarify the anti-inflammatory effect of PL on persistent synovitis, we identified the biomarkers that reflect the inflammatory state of the equine persistent synovitis model induced by monoiodoacetic acid (MIA). Thereafter, we evaluated the changes in these biomarkers after repeated intra-articular administration of PL in this model.

METHODS: (Expt 1) Synovitis was induced by administering MIA (0.18 mg/kg body weight) into the unilateral antebrachiocarpal joints of five horses and saline was injected into the contralateral joints as control on day 0. Clinical and ultrasound examinations and synovial fluid collection were performed on days 0, 1, 2, 7, 14, 21, 28, and 35. Lactate dehydrogenase (LDH), interleukin-1 receptor antagonist (IL-1Ra), and transforming growth factor- β 1 (TGF- β 1) levels in the synovial fluids were measured. Synovium was obtained after euthanasia on day 42, and gene expression of inflammatory biomarkers in tissues was quantified by real-time PCR.

(Expt 2) As in Expt 1, synovitis was induced by administering MIA in both the legs of six horses on day 0. PRP was prepared by a double-spin method and freeze-thawed twice. Thereafter, the centrifugation supernatant was collected to prepare PL. PL (2 mL) was administered into the unilateral antebrachiocarpal joints and saline into the contralateral joints on day 21, 28, and 35. Clinical and ultrasound examinations and synovial fluid collection were performed just before the PL administration. LDH and TGF- β 1 concentrations in the synovial fluids were measured as in Expt 1. Synovium was obtained on day 42, and gene expression was quantified similar to that in Expt 1.

RESULTS: (Expt 1) Lameness score and IL-1Ra returned to the control levels by day 28, but some indicators of chronic inflammation remained high until day 35. Expression of matrix metalloproteinase 13 (*MMP13*), a disintegrin and metalloproteinase with thrombospondin motifs 4 (*ADAMTS4*), and receptor activator of nuclear factor kappa-B ligand (*RANKL*) was significantly higher in MIA model than in control. (Expt 2) PL and saline groups showed no difference in all the high marker values in the MIA model in Expt 1.

DISCUSSION & CONCLUSIONS: The persistent synovitis model induced by MIA exhibited characteristics of chronic inflammation. However, PL did not exert an anti-inflammatory effect. Further studies may be needed to determine the appropriate intra-articular PL dose and administration frequency.

Keywords: Cartilage / joint and arthritic conditions, In vivo and animal models

PP-252

Donor-dependent proteome variation in equine platelet-rich plasma

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INTRODUCTION: Platelet-rich plasma (PRP) is used as an autologous regenerative therapy of musculoskeletal disorders, with varied clinical effects. It is unclear how donor characteristics affect protein composition of PRP, which may impact on clinical outcomes. This study investigated potential differences in the abundance of proteins in equine PRP by donor age, sex and presence of musculoskeletal injury.

METHODS: Following ethical approval (VREC980a) and informed owner consent, blood samples were obtained from horses: i) admitted for treatment of tendon or ligament injury with autologous PRP; ii) healthy horses undergoing routine surgery under general anaesthesia. PRP was prepared using a commercial filtration kit (V-PET, Pall), platelet (PLT) and white blood cell (WBC) counts determined in a subset of samples, prior to storage at -80°C. PRP proteins were identified using label-free liquid chromatography-tandem mass spectrometry, following protein equalisation (ProteoMiner, Bio-Rad), and MaxQuant software with 1% false discovery rate. Downstream analyses were performed in R. Exploratory data analysis was undertaken using Principal Component Analysis (PCA). Secreted proteins are considered to mediate the therapeutic effect of PRP, therefore, further statistical analysis was performed on proteins annotated as extracellular according to the UniProt database. Generalised Linear Models (GLM) were used to test the relationship between extracellular protein abundance and horse injury status, age and sex. Results were corrected for multiple comparisons (Benjamini-Hochberg method) and significance assumed if adjusted P-value <0.05.

RESULTS: Nineteen healthy horses (age 2-16 years, mean 8.7) and 9 injured horses (age 4-16 years, mean 11.4) were included. Mean PLT was 671x103/μL (SD 157.6 x103) and mean WBC 11.2 x103/μL (SD 3.7 x103). Across all samples, 453 proteins were identified based on the presence of ≥2 unique peptides. PCA separated the samples into two moderately discrete clusters along PC2, corresponding with presence/absence of injury. GLM of extracellular proteins (109) demonstrated that abundance of 15 proteins was affected by the presence of injury, but not donor age or sex. PRP from injured horses showed higher abundance of proteins related to innate immunity and vitamin binding.

DISCUSSION & CONCLUSIONS: Results suggest that presence of injury increases immunostimulatory effect of PRP, in agreement with previous work suggesting innate immune response activation in horses with tendon injuries [1]. These findings raise awareness for considering potential clinical implications of using autologous PRP to treat musculoskeletal injuries.

ACKNOWLEDGEMENTS: This study was funded by Horserace Betting Levy Board Equine Post-Doctoral Fellowship (VET/2020-2EPDF8)

REFERENCES: 1. Turlo et al. 2015. PLoS ONE;10:e0140673

Keywords: Omics / bioinformatics and systems biology, Veterinary

PP-253

Electrospun Collagen Scaffold Bio-Functionalized with Recombinant ICOS-Fc: An Advanced Approach to Promote Bone Remodelling

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INTRODUCTION: Osteoporosis (OP) derives from an imbalance between bone resorptive cells, osteoclasts, (Oc) and bone-forming ones, osteoblasts, (Ob) resulting in low bone density, thus fractures. This study presents a smart-treatment strategy for osteoporotic fractures, especially those not surgically treatable due to their anatomical location (e.g. pelvic fractures) and the frailty context of the patient. This approach is based on the development of an electrospun resorbable and flexible scaffold which can be injected at the fracture site. This platform should allow for Obs to proliferate and grow whilst reducing the action of Oc, resulting in an overall boost to the hampered bone regeneration. For that, type I collagen-based fibrous flexible matrices have been produced by an optimized electrospinning process, and subsequently cross-linked and bio-functionalized with ICOS-Fc, a recombinant molecule able to reversibly inhibit human Oc activity when bound to cell surface receptor ICOS-L [3].

METHODS: Type I collagen was extracted from rat tail tendons, dissolved in acetic acid at a concentration of 40 wt%, and electrospun using a flat collector. The obtained electrospun membranes were then soaked in an ethanol solution of EDC and NHS, also containing ICOS-Fc (50 µg/mL), to simultaneously promote cross-linking and biofunctionalization of collagen fibres. ELISA-like assay with ICOS-L as capture confirmed the functionality of the protein. Cytocompatibility was studied with MC3T3-E1 cells while U2OS (ICOSL positive) and HOS (ICOSL negative) were used for migration assays.

RESULTS: SDS-PAGE and CD analysis proved the preservation of the supra-molecular structure of collagen and FESEM micrographs revealed micro-nanofibrous electrospun scaffolds (fibre diameter ca 120 nm), maintained after crosslinking. ELISA-like assay confirmed ICOS-Fc grafting onto collagen fibres and the retention of the ability to bind its ligand. The matrices were cytocompatible and functionalised ones hindered cell migration on ICOC-Fc positive cells (at variance with ICOS-Fc negative cells).

DISCUSSION & CONCLUSIONS: Collagen-based electrospun flexible scaffolds have been produced by an optimized electrospinning process and treated with EDC/NHS ethanol solution containing ICOS-Fc biomolecule, permitting simultaneous crosslinking and biofunctionalization. The resultant scaffolds were able to reduce the migration of osteoclast-like cells proving to be an important strategy for bone tissue engineering.

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2. Coughlan, T. et. al. Clin. Med. 2014, 14, 187–191.

3. Gigliotti, C.L. et. al. J. Immunol. 2016, 197, 3905–3916.

Keywords: Biofabrication, Biomaterials

PP-254

Incorporating Strontium enriched amorphous calcium phosphate microparticles in collagen/Magnesium-hydroxyapatite osteochondral scaffold improves subchondral bone repair

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INTRODUCTION: An osteochondral scaffold with a superficial collagen layer for cartilage replacement and a collagen/Magnesium-hydroxyapatite (Col-Mg-HAp) layer for bone replacement has demonstrated good clinical results in osteochondral defects. However, subchondral bone repair remained suboptimal compared to the cartilage repair. Calcium phosphates have been reported to possess osteoconductive and osteoinductive characteristics. In addition, Strontium (Sr) was demonstrated to promote bone formation. In this study, Sr enriched amorphous calcium phosphate (ACP) microparticles were incorporated in the Col/Col-Mg-HAp scaffold, and the effectiveness of ACP/Sr-ACP incorporation on bone repair in osteochondral defects was evaluated in vivo.

METHODS: ACP/Sr-ACP was processed into granular form (100-150 µm) using a dry granulation technology. Phase composition of the granules was analysed using x-ray diffraction. Information about chemical groups was gathered using Fourier-transform infrared spectrometer. Then 30 w/w% ACP/Sr-ACP microparticles were integrated into the scaffold prototypes. To characterize the scaffold prototypes, µCT and scanning electron microscopy (SEM) were used. To study the effect of ACP/Sr-ACP on bone repair at the early phase, osteochondral defects were created in bovine osteochondral biopsies, filled with scaffolds and implanted subcutaneously in 12-week-old athymic mice for 8 weeks. Histology was used to evaluate defect filling with osteochondral tissue. To further investigate the osteogenic effect of Sr-ACP, critical-sized defects were created in the medial femoral condyle in 8 goats' stifle joints and filled with Sr-ACP enriched scaffolds. After 6 months defect repair was evaluated with µCT and histology.

RESULTS: The amorphous character and the hydrated and carbonated nature of ACP and Sr-ACP granules were confirmed. Specific surface area of both granules was high (> 100 m²/g). The Sr-ACP granules were well distributed and embedded in the collagenic matrix demonstrated by µCT and SEM. After 8 weeks in mice, slightly more osteochondral tissue was found in the defects loaded with Sr-ACP enriched scaffolds (89.3±7.2%) compared to the ACP enriched scaffolds (80.2±21.5%) and scaffold-only (87.2±11.1%). After 6 months in goats, all the defects were classified as nearly normal cartilage macroscopically (grade II, International Cartilage Repair Society scores). µCT analysis demonstrated good healing in the bone defects. Histologically, significantly more bone was observed in the bone defects loaded with Sr-ACP enriched scaffold compared to scaffold-only (88.8±7.6% vs.

77.3±11.3%, P=0.038).

DISCUSSION & CONCLUSIONS: The addition of Sr-ACP-microparticles is promising to further improve the bone formation capacity of a Col/Col-Mg-HAp scaffold. A longer follow-up study is needed to demonstrate the long-term improvement of this scaffold in osteochondral repair.

Keywords: Cartilage / joint and arthritic conditions, In vivo and animal models

PP-255

3D printed hybrid for articular cartilage regeneration: from design to *in vivo* studies

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INTRODUCTION: Articular cartilage is the bearing surface between opposing bones in joints. It is characterised by a viscoelastic behaviour and a smooth outer surface, but also by limited self-repair ability. A chondral implant to regenerate articular cartilage was designed, based on an innovative silica/polytetrahydrofuran/polycaprolactone (SiO₂/PTHF/PCL-diCOOH) sol-gel hybrid. This material can be 3D printed into porous scaffolds with controlled porosity and tuneable mechanical properties [1]. The aim was to develop a chondral device that can improve microfracture surgery by bonding a bulk articular surface to a 3D printed scaffold. Challenges are: 1) achieving a successful bond of the two components; 2) having the lowest possible friction coefficient against living cartilage; 3) guidance of human bone marrow stem cells (hMSCs) to produce high quality articular cartilage.

METHODS: SiO₂/PTHF/PCL-diCOOH hybrid sols were prepared combining the silica sol-gel process with in situ THF polymerisation [1]. The sol was cast into moulds to obtain the dense cap and 3D printed to produce the porous scaffold. The two components were joined when still wet, then aged and dried. The device 3D structure, mechanical and tribological properties, in vitro and in vivo biological behaviour were evaluated.

RESULTS: The device showed high flexibility with ability to recover the deformation under cyclic loading, matching the mechanical properties of native cartilage. Excellent fusion between the scaffold and the cap was observed under the SEM and digital image correlation. No signs of wear were detected when tested against natural cartilage in physiological conditions. Cartilage cells and commercially available hMSCs seeded on the scaffolds demonstrated that channels of ~200 µm promote excellent cartilaginous matrix formation *in vitro* following chondrogenic differentiation. Three implants were investigated *in vivo* using an ovine model: 200 µm channel size scaffolds; 500 µm channel size scaffolds; the devices with a bulk cap and 200 µm channel size scaffold. Press-fitting the implants with the addition of cyanoacrylate glue was used as the fixation method, as well as a periosteal flap for the scaffolds to promote lubrication.

DISCUSSION & CONCLUSIONS: Mechanical testing, tribological evaluation, *in vitro* and *in vivo* studies allowed to identify the best solution for articular cartilage regeneration, with signs of collagen type II formation and proteins such as proteoglycans.

ACKNOWLEDGEMENTS: The work was supported by EPSRC grant EP/N025059/1 and the NIHR ProTECT grant from NIHR, UK.

REFERENCES: [1] Tallia F. et al., Materials Horizons, 2018, 5, 849-860

Keywords: Additive manufacturing, Cartilage / joint and arthritic conditions

PP-256

Synthetic Collagen Peptides for Investigating the Role of Specific Cell Adhesion Interactions in 3D Biomaterials

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INTRODUCTION: Collagen is the most ubiquitous biomacromolecule found in the animal kingdom and is commonly used as a biomaterial in regenerative medicine therapies. Cells bind to collagen through a variety of adhesion motifs which can also influence gene expression through the regulation of intracellular signalling pathways, such as the SMAD complex [1]. Our hypothesis is that collagen-based biomaterials may be augmented by specifying which adhesion motifs cells utilise during *in vitro* studies. Here we demonstrate the design of synthetic collagen peptides with specific binding affinity to $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins, as well as discoidin domain receptors 1 and 2 (DDR1/2). We also demonstrate their incorporation into viscoelastic 3D hydrogels suitable for 3D bioprinting.

METHODS: Synthetic collagen peptides with different central 'guest' sequences including GROGER and GVOGEA were commercially produced using F-moc synthesis (Genscript, USA). Circular dichroism (CD) spectroscopy was used to measure the peptide ellipticity between 190–270 nm, and measure the peptide melting temperatures. Human mesenchymal stem cell (MSC), foreskin fibroblast and fibrosarcoma cell lines were cultured in 2D on peptide coatings in the presence of $\alpha 1$, $\alpha 2$ and $\beta 1$ integrin subunit function blocking antibodies in order to verify the peptide-binding site specificity. Peptides were also functionalised to gellan gum hydrogels using carbodiimide chemistry, in order to create a biomaterial suitable for 3D extrusion bioprinting.

RESULTS: Molar ellipticity spectra showed a triple helical conformation for all collagen peptide designs. CD spectroscopy was also used to measure the melting temperature of each peptide, which were all sufficiently high (52°C - 64°C) to prevent helical unfolding at physiological temperatures. Fibrosarcoma cell adhesion was only observed when adhering to GROGER peptide designs, confirming that this sequence binds specifically to $\alpha 2\beta 1$ integrin. Inhibition of fibroblast and MSC binding to the GVOGEA peptide when incubated with inhibitory antibodies confirmed the binding specificity of this sequence to $\alpha 1\beta 1$ integrin. Rheological characterisation of peptide-functionalised gellan gum hydrogels confirmed that this biomaterial maintained its viscoelastic properties post cross-linking.

DISCUSSION & CONCLUSIONS: Triple helical collagen peptides capable of binding to specific integrins were successfully produced. Peptide-functionalised hydrogels were also successfully created, enabling the investigation of specific matrix cues on cell phenotype in 3D. We intend to utilise this model to investigate the role of individual binding mechanisms to improve the tri-potential differentiation of MSCs to aid in their potential for regenerative medicine research.

REFERENCES: [1] Lian et al, Collagen type II suppresses articular chondrocyte hypertrophy and osteoarthritis progression by promoting integrin $\beta 1$ -SMAD1 interaction, Bone Res. 7 (2019).

Keywords: Biomaterials, Microenvironment and niche engineering

PP-257

Peptide hydrogels for 3D hepatocyte encapsulation and function

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INTRODUCTION: Liver diseases are a significant public medical burden[1]. To overcome the lack of donors and high cost of therapies there is a need to develop long-term culture systems that maintain hepatocyte cell viability and specific functions. We exploit a novel self-assembling peptide hydrogel (SAPH) that can mimic the normal hepatic extracellular matrix and lead to hepatic cellular function. Indeed, previous reports[2] have demonstrated that 3D cell culture allows hepatocytes to maintain differentiation and function.

METHODS: All tested self-assembled peptide hydrogels, PeptiGel® Alpha1, Delta1, Alpha2, Alpha3, Alpha4, Delta5, Alpha6 and Alpha7 were purchased from MANCHESTER BIOGEL. HepG2 cells (1×10^6 /ml) were encapsulated within SAPHs. The theoretical net charge was calculated, and viscoelastic properties of peptides were investigated using rheometry. The Cell viability over 14 days culture was investigated using LIVE/DEAD® cell viability assay with confocal microscopy. Albumin production and urea secretion were tested using Human Albumin ELISA Kit (ab179887) and Urea Assay Kit (ab83362). qPCR assay was used for measuring Cytochrome P450 enzyme activities over 14 days culture and detecting drug induced CYP450s mRNA level. Immunohistochemical staining was used to observe the ECM deposition, Albumin production, and E-cadherin cell-cell interactions of HepG2 cells encapsulated within peptide gels.

RESULTS: A cell viability assay showed that over 14 days in vitro culture, HepG2 cells proliferated within Delta1, Delta5 and Alpha7, and could survive within Alpha1, Alpha2, Alpha3. ELISA assay revealed a significant increase of Albumin production; and HepG2 cells maintained urea secretion which increased after day7. qPCR determined increased level of RNA expression for CYP1A2, CYP2C9, CYP3A4 and Albumin, from day1 to day14; detected CYP2E1 as the main metabolic gene for acetaminophen, was significantly induced, after exposure to acetaminophen 48 hours at day9. Cells produced fibronectin, laminin, collagen I, intercellular albumin and E-cadherin according to histology staining.

DISCUSSION & CONCLUSIONS: Hepatocytes could maintain cell viability and proliferation when encapsulated within negative-charged peptides rather than positive-charged peptides. Hepatocytes can maintain the main hepatic functions within Alpha7 over 14 days and also drug metabolism activities in response to acetaminophen. These results suggest that Alpha7 has potential for liver tissue engineering applications, the study of hepatocytes in 3D culture and also as an in vitro model for drug toxicity prediction.

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Keywords: Hydrogels and injectable systems,

PP-258

Tissue constructs with Mesenchymal stromal cells and TGF- β 3 encapsulating scaffolds with potential use in skin wound repair.

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INTRODUCTION: The skin is the body's largest organ and, as such, has a fundamental role in protection against mechanical forces, infection, fluid imbalance, and thermal dysregulation. It also provides flexibility and elasticity that allow the joints to function properly. After a thermal or traumatic injury, the skin repair process is characterized by a fibroproliferative dysfunction, causing excessive deposition and changes in the morphology of collagen and another extracellular matrix (ECM) proteins, leading to functional and aesthetic tissue problems. To address this problem, the anti-fibrotic growth factor TGF- β 3 was incorporated into calcium alginate capsules and immobilized between a poly(ϵ -caprolactone)/collagen type I scaffold obtained by electrospinning in a sandwich-like structure (PCAT).

METHODS: In order to evaluate the TGF- β 3 release profile in both the capsules and the PCAT, an enzyme-linked immunosorbent assay (ELISA) was performed. In addition, in vitro characterization was performed to assess the cytocompatibility and bioactivity of PCAT using green fluorescent protein-labeled human Wharton's jelly mesenchymal stromal cells (GFP-hWJ-MSCs). A bead-based immunoassay was also used to assess PCAT-modulated skin tissue repair-associated growth factors in hWJ-MSCs.

RESULTS: We found that calcium alginate capsules allowed for the progressive release of TGF- β 3 for up to eight days, with zero order release kinetics, indicating the sustained presence of the protein over time. The fibroblast-like morphology of GFP-hWJ-MSCs confirms the cytocompatibility of the scaffold, and the proliferation assay shows that TGF- β 3, a mitogenic factor, increases cell proliferation. These results show that the encapsulation of TGF- β 3 in calcium alginate preserves its bioactivity and ensures its bioavailability over a long period of time. PCAT increased secretion of TGF- β 2 and FGFb, maintained PDGF-AA and HGF, and decreased expression of VEGF-AA by hWJ-MSCs, thereby regulating the production of growth factors associated with skin tissue repair.

DISCUSSION & CONCLUSIONS: In this study, PCAT scaffolds were viable for generating a construct with hWJ-MSCs. The results suggest that the generated constructs may be potential skin substitutes for long-term skin lesion treatment.

Keywords: Biomaterials, Wound healing

PP-259

Mechano-Hybrid-Scaffolds for endochondral healing of bone defects

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INTRODUCTION: Collagen scaffolds are well known for their regenerative potential and are bio-mimicry of early callus formation. However, due to their relatively poor mechanical properties, their applications in the field of bone regeneration are limited. In this study, we present a novel approach: A Mechano-Hybrid-Scaffold (MHS) that combines a collagen-based biomaterial with highly aligned channel-like pores with a 3D printed poly(ϵ -caprolactone) (PCL) support structure [1], overcoming contradictory requirements for mechanical stiffness and scaffold architecture on the cell vs. the tissue length-scale that are defined by the particular clinical application.

METHODS: Using a standardized manufacturing process including directional freezing the existing orientated collagen scaffold [2] (stiffness $E = 0.5$ kPa) was combined with a 3D printed PCL macro-porous support structure that was designed to reinforce the scaffolds mechanical stability. In brief, MHS were produced by: 1. Computer-aided design of the support structure (2 alternative designs for high stiffness "stiff" and low stiffness "soft"); 2. Selective laser sintering from PCL; 3. Immersion of the support structure into the collagen dispersion, unidirectional freezing and freeze-drying; 4. Chemical crosslinking and sterilization of the MHS. Structural characterization (SEM) and mechanical characterization (BOSE Electroforce test bench) were performed. Degradability of the MHS was assessed by determination of the denaturation temperature of the collagen (Td).

RESULTS: Stiff and soft MHS were successfully produced with stiffness of 41.2 ± 1.4 N/mm and 0.1 ± 0.02 N/mm for the supporting structure respectively. Except for the mechanical properties of the MHS, all other scaffold characteristics, e.g. Td ($79.8^\circ\text{C} \pm 0.1$) and pore size ($78.1 \mu\text{m} \pm 18.1$), remain the same as the ones of the collagen scaffold. Scanning electron microscopy (SEM) images show full integration of the support structure inside the collagen structure. With this approach, mechanical characteristics can be tuned independently at the micro-scale (cell-level) and the macro-scale (tissue-level).

DISCUSSION & CONCLUSIONS: The here-introduced concept takes full advantage of the regeneration potential of collagen scaffolds by implementing a second macro-porous structure that provides mechanical support against cell and tissue forces in combination with clinically applied bone fixation systems. The MHS opens the door for new applications of collagen scaffolds in rigid tissue regeneration by solving the paradox of providing soft cell environment and high structural stability in implantable materials.

ACKNOWLEDGEMENTS: German Federal Ministry of Education and Research (BMBF) via grants no. 13XP5048A-D).

REFERENCES: [1] DE102016007931.2; PCT/DE2017/000183

[2] Petersen et al. (2018), Nat. Commun. 9:4430.

Keywords: 3D printing and bioprinting, Biomaterials

PP-260

Testing of Polyhydroxyalkanoate films for breast cancer models

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INTRODUCTION: Polyhydroxyalkanoates (PHA) are a family of natural biodegradable polyesters that have been tested for a variety of biomedical applications such as tissue repair and drug delivery. It is the aim of this study to evaluate novel PHA blends, consisting of medium chain-length polyhydroxyalkanoates (mcl-PHA), an elastomeric version of PHA; and Poly(3-hydroxybutyrate) (PHB) a rigid version of PHA, as substrate for the culture of breast cancer cell lines towards the development of an in vitro breast cancer model.

METHODS: Films of PHA blends containing various ratios of mcl-PHA and PHB such as 100:0, 75:25, 50:50, 25:75 and 0:100, were produced by the solvent-casting method. The films were physically characterised by scanning electron microscopy (SEM), tensile testing, thermogravimetric analysis (TGA), Fourier-transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC) and X-ray diffraction (XRD). The behaviour of the MDA-MB-231 breast cancer cell line was analysed by a migration assay using the fluorescent ubiquitination-based cell cycle indicator (FUCCI), a live-dead assay and a WST-8 growth assay.

RESULTS: SEM images indicated the surface roughness and porosity of the films. Tensile testing indicated that increasing the ratio of PHB resulted in increased stiffness of the films. The thermal properties of the blends are also changing with different PHA species ratios, as well as crystallinity, compared to the neat polymers. A higher rate of cell growth of FUCCI-transfected MDA-MB-231 was observed on the PHA blends with increasing stiffness with higher PHB content. Ultimately, a high rate of living cells and cell migration over six days was observed on all films.

DISCUSSION & CONCLUSIONS: An increased cell growth rate has been observed on the blends with increasing film substrate stiffness. This study helps determine the optimal PHA blend for further generation of 3D in vitro breast cancer models using sustainable bacterial-derived polymer. Further fabrication methods will be explored as next step to for an ideal breast cancer model development.

Keywords: Polymers - natural / synthetic / responsive, Cancer

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Porous Ca-P/PLA Composite Modified with Alendronate Exhibits Enhanced Potential for Bone Tissue Regeneration

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INTRODUCTION: The clinical, safe and effective treatment of large bone defects (caused by tumor resection, osteoporosis or trauma) is still a frequently discussed topic. Synthetic implants used to treat large bone defects are often unable to regenerate. It is necessary an application of synthetic materials which exhibit biocompatibility and resorbability, for example polymer-ceramic composites (polylactide and calcium phosphate Ca-P). However the composites often require additional modification due to obtain appropriate enhanced properties, especially in mechanical and biological aspect. In the case of materials for bone defects regeneration the very important feature is their feasibility of osteoinductive activity. Sodium alendronate (ALN), the first-line drug for osteoporosis, binds to hydroxyapatites in the bones, and thus inhibits the activity of osteoclasts, which cause bone tissue resorption and does not inhibit the bone formation process in the same time.

METHODS: The porous Ca-P/PLA composite was obtained by using a thermally induced phase separation method. As the filler of composite, Ca-P whiskers with silane proadhesive agent on the surface was used. Alendronate modification of the composite was carried out by adsorbing of ALN on the surface of the composite. The obtained composite was characterized in respect of physicochemical, mechanical and biological properties.

RESULTS: Alendronate modification was carried out successfully and the obtained composite was characterized by a microstructure appropriate for porous materials dedicated to bone fillings, mechanical strength at the level of a natural cancellous bone and the ability to release an active substance. The composite was non-cytotoxic towards L929 and hFOB 1.19 cell lines, and they didn't activate the NF-κB signaling path-way in human THP1-Blue™ NF-κB monocytes. The composite with ALN increased the proliferative potential and enabled cell growth of human fetal osteoblasts (hFOB 1.19) compared to the control material lacking alendronate. The presence of ALN stimulated osteoblasts to differentiate (significant increase in OC and ALP production).

DISCUSSION & CONCLUSIONS: The modification of the composites with alendronate resulted in efficient scaffold colonization, which in turn would be expected to translate to high-density bone tissue formation and an enhanced regenerative potential in such composites.

The obtained composite seems to be a very promising candidate suitable for future biomedical engineering applications.

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Keywords: Composite materials, Biomaterials

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How to create the ideal GelMA formulations for specific applications

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INTRODUCTION: Gelatins are widely used in the biomedical and tissue engineering fields, due to their biocompatibility, biodegradability and cell-interactivity. Gelatin can be chemically crosslinked to originate stable hydrogel constructs at body temperature. However, to achieve predictable hydrogel strengths several factors need to be acknowledged, such as consistent production. Important is also the resin formulation, starting from consistent molecular weight (MW), degree of modification (DoM), salt concentrations, polymer concentration, photo-initiator (PI) concentrations, and temperature control in the pre-phase and during hydrogel production. The interplay is diverse, but mappable and hence insightful decisions can be made early on in the experimentation process.

METHODS: Six GelMA types (90 kDa and 160 kDa at DoM of 40, 60 and 80 %), were dissolved at various concentrations in various types of PBS. Various photo-initiators were researched at various concentrations in the GelMA resin formulations. The crosslinking kinetics and the hydrogel strengths of the GelMAs were studied at various temperatures using photo-rheology, tensile testing, compression testing, and dynamic mechanical analysis.

RESULTS: The herein presented data confirm that with increasing degree of modification (DoM) the storage modulus of the GelMA hydrogels will increase too. The increase in DoM can be correlated to an increase in storage modulus with an exponential component of $e^{\sim 0.6x}$ for the 160 kDa GelMAs and an exponential component of $e^{\sim 0.54x}$ for the 90 kDa GelMAs. With increasing GelMA concentrations (w/v%) the storage modulus of the hydrogel will increase. For the 90 kDa GelMAs a near perfect power-correlation ($x^{\sim 3.3}$) was established. The 160 kDa GelMAs showed a linear correlation. The salt concentration of the solvent affects the hydrogel strength with a variability ranging over 90 % a difference. The temperature at which photo-crosslinking is performed causes a 95 % difference in hydrogel strength depending on, the temperature in the preparatory phase, the concentrations and types of PI, the MW, the DoM, and GelMA concentration.

DISCUSSION & CONCLUSIONS: With consistent GelMA molar mass and degree of modification, hydrogel strength becomes predictable. However, the applied protocols greatly affect hydrogel outcomes, as demonstrated through salt concentrations, PI concentrations, sterilization processes and the temperatures applied in the preparation and the curing phases. In short, the interplay between the various components of a GelMA resin is diverse, but it is mappable and hence insightful decisions can be made, greatly improving the fruits of your labor.

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Keywords: Biomaterials, Biofabrication

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The development of a responsive supported lipid bilayer coating to prevent uncemented joint replacement infections

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INTRODUCTION: Infection is the third leading cause of joint replacement failure, which requires revision surgery. Supported lipid bilayers (SLBs) with encapsulated antimicrobial agents offer an exciting approach to prevent bacterial infections on uncemented implants. In this study, titanium was functionalised using octadecylphosphonic acid (ODPA) for the subsequent formation of an SLB with encapsulated novobiocin, an aminocoumarin antibiotic effective against *Staphylococcus aureus*.

METHODS: The SLB with encapsulated novobiocin was developed on the surface of ODPA-modified TiO₂-coated quartz crystal microbalance (QCM) sensors using a solvent assisted method (SALB)¹. The obtained SLB was analysed by QCM and fluorescence microscopy using fluorescent lipids. The prototype implant surface was tested for antimicrobial activity against *S. aureus* and cytotoxicity tested against human bone marrow stromal cells (hBMSC) using a CCK-8 assay. The SLB was loaded with a fluorescent probe (6-FAM) and incubated with α -hemolysin (0.1-10 μ g/mL), a pore-shaped *S. aureus* virulence factor, to study triggered release mechanisms.

RESULTS: SLB fabrication with QCM monitoring revealed that shifts in resonance frequency (ΔF) (-68 Hz) and the energy dissipation (ΔD) (28×10^{-6}) support the successful formation of a planar SLB with encapsulated novobiocin using the SALB, which was also confirmed by fluorescence microscopy. Incorporation of novobiocin in the SLB resulted in reduced attachment and viability of *S. aureus*, without significant reductions in hBMSC viability. Low levels of 6-FAM were released in a dose-dependent manner when the SLB was incubated with α -hemolysin.

DISCUSSION & CONCLUSIONS: In the present study, an SLB was developed on the surface of titanium that was triggered to release encapsulated contents by a bacterial virulence factor, α -haemolysin. Similar studies have demonstrated this approach is feasible for lipid nanoparticles², however this is the first study to demonstrate a triggered release from the surface of a metal. The findings indicate the possibility of creating a biocompatible metal implant coating that releases an antimicrobial in the presence of pathogens in a dose dependent manner. Further optimisation is required to achieve therapeutic levels of antimicrobial release. Such an approach has the potential of reducing implant infections, whilst preventing the likelihood of antimicrobial resistance or cytotoxicity.

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Keywords: Infection, Drug delivery

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Analysis of particle recognition and uptake by osteoblasts following short-time exposure to metallic particles

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INTRODUCTION: Aseptic loosening is the main risk factor for the failure of endoprosthetic implants. In this process, metal particles and their corrosion products can trigger a cellular inflammatory reaction leading to osteolysis. Osteoblasts are among the cells exposed to wear particles at a very early stage. However, the underlying mechanisms of cellular interaction with particles have not been fully elucidated. Therefore, the recognition and uptake of metallic particles by primary human osteoblasts (hOBs) were investigated using hyperspectral darkfield microscopy. Further, we determined temporal gene expression profiles of Toll-like receptors (TLR) and associated intracellular signaling pathways to evaluate the activation process after particle contact.

METHODS: HOBs (n=6) were treated with cobalt-chromium-molybdenum (CoCr28Mo6) particles (0.01 mg/ml) with an average size of 1.6 µm. Furthermore, hOBs exposed to lipopolysaccharides (LPS; 1 µg/ml) were included as the positive control. After incubation times ranging from 10 minutes to three days, gene expression of *TLR2*, *TLR4*, *interleukin (IL)-1β*, *tumor necrosis factor-alpha (TNF-α)*, *caspase (Casp) 1*, and *Casp 4* were examined as markers of particle recognition and uptake. In addition, cell morphology and particle incorporation were examined using hyperspectral darkfield microscopy. The data were validated by analyzing the same cells using scanning electron microscopy and Energy Dispersive X-Ray (EDX) analyses.

RESULTS: The microscopic examinations revealed that the human osteoblasts took up the first particles intracellularly after only 10-30 minutes. Over time, more particles were taken up and agglomerated within the cells. The hyperspectral data of the particles showed a shift of the spectra to higher wavelengths with increasing time, so it can be assumed that particle phagocytosis occurred. Particle recognition and uptake were also reflected in the gene expression profile. The first changes in mRNA levels were detected after 1 h of particle exposure. Initially, an increase in *IL-1β* expression was shown, which significantly increased after treatment with LPS. The expression rates of *TLR4*, *CASP4*, and *IL-1β* increased continuously from the 4 h particle exposure to over 72 h, whereas the expression of *TLR2* peaked at 24 h. The expression of *CASP1* increased between 8 and 16 h and decreased again in the further course.

DISCUSSION & CONCLUSIONS: HOBs can recognize, phagocytose, and respond to metallic particles after a short-term exposure by initiating a pro-inflammatory response. The immediate response is associated with TLR signaling. With increasing time, there is an activation of the inflammasome, thus, amplifying the inflammatory response via increased secretion of IL-1β.

Keywords: Bone and bone disorders (osteoporosis etc), Immunity / immunomodulation / macrophage

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3D printed bone grafts: Toughness enhancement utilizing PCL-based additives

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INTRODUCTION: One main goal of bone tissue engineering (BTE) is the replacement of autografts with artificial bone scaffolds as a treatment method for major bone defects. Alternatives to traditional manufacturing methods are needed to meet patient- and defect-specific requirements. One attractive technique for this is digital light processing stereolithography (DLP-SLA). Showing good biocompatibility and low cytotoxicity, vinyl esters (VEs) are promising monomers for BTE applications. Nevertheless, crosslinked VE-thiol networks show very brittle behavior. To overcome this obstacle and decrease brittleness, poly(ϵ -caprolactone)-derived (PCL) high-molecular weight additives might be utilized.¹ This approach was further investigated, and a library of PCL-based additives was synthesized and tested. In addition, a rigid thiol compound was introduced and compared to a flexible commercially available one.²

METHODS: Additives and thiols were tested in formulations containing divinyl adipate (DVA) as matrix monomer. Two different toughness enhancing additives (TEAs) containing photopolymerizable end-groups (allyl carbonates) with different molecular weights and two thiols were examined. Their impact on photoreactivity was tested using real time-near infrared photorheology. (Thermo-)Mechanical properties were investigated using dynamic mechanical thermal analysis and tensile testing. As a proof-of-concept, surgical screws were 3D printed with the best-performing formulation. A 3D scan of the printed object was overlayed with the original CAD file to investigate the dimensional accuracy of the printed parts.

RESULTS: Photoreactivity studies showed that upon addition of TEAs, the time until gelation t_{gel} was reduced (minimum t_{gel} was 2.6 s) and an increase in the double bond conversion (DBC) compared to reference formulations without TEA was found. DBCs up to 86 % were observed. Glass transition temperatures were above 37 °C, which is crucial for BTE applications. Tensile testing revealed a substantial increase in mechanical properties: it was possible to triple the materials' tensile toughness. 3D printed parts exhibited low volumetric shrinkage between 100-200 μ m and neither cracks nor delamination.

DISCUSSION & CONCLUSIONS: The addition of PCL-based TEAs led to an outstanding increase in mechanical properties, especially regarding tensile toughness, without disrupting photoreactivity. Thus, VE-based systems containing TEAs provide an excellent alternative to state-of-the-art (meth)acrylate-based systems.

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Keywords: Additive manufacturing, Polymers - natural / synthetic / responsive

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Designing a Peptide Hydrogel for Cancer Early Detection

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INTRODUCTION: Cancer early detection (CED) is pivotal to patient survival. The small non-coding nucleic acid sequences, microRNA (miRNA) are a captivating molecular target for CED. miRNA are dysregulated during the early stages of cancer¹, it is found in stable amounts in blood plasma and serum. Therefore, a minimally invasive liquid biopsy screening device would allow for point of care diagnostics. Current miRNAs detection methods are cumbersome and lack reproducibility. To overcome these challenges we aim to develop a new diagnostic platform using a functional 3D peptide hydrogels for sequence specific^{2,3}, PCR-free, fluorescent detection of miRNAs in a “one-pot” assay.

METHODS: A split probe system with a FRET pair is loaded within an anti-parallel β -sheet peptide hydrogel, this allows complementary strand of cancer miRNA biomarkers to be identified via fluorescence. Diffusion characteristics evaluated via plate reader and Fluoroblok well insert to measure the rate at which fluorescently labelled DNA analogue of miR-21 diffuse into peptide hydrogels. Cell culture of Panc-1, and MIA PaCa-2 in 2D and 3D (multicellular tumour spheroids) forms was undertaken to validate the sensor.

RESULTS: Four de novo designed self-assembling peptide hydrogels (SAPH) were tested to understand the diffusion characteristics of miRNA and select system that allow fast trapping and detection of miRNA.

DISCUSSION & CONCLUSIONS: Positively charged hydrogels promoted miRNA trapping due to the electrostatic attraction. The mesh size of the hydrogel used (<40nm) also allowed to filter and avoid interference from larger cell debris usually present in biological samples. The SAPH provides a 3D platform for cancer cell culture, to validate the sensor with real-type miRNA, while also revealing how the materials physical characteristics influences the migration behaviour in pancreatic cancer.

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Keywords: Hydrogels and injectable systems, Cancer

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Enzyme-Responsive Hydrogels As Skeletal Muscle Implants

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INTRODUCTION: Volumetric muscle loss (VML) is a common condition caused by traumatic or surgical loss of skeletal muscle tissue.¹ VML activates an inflammatory response in muscular tissues, which triggers the upregulation of lysyl oxidase (LOX), an enzyme that catalyses collagen crosslinking via Schiff base chemistry. LOX oxidises primary amines of collagen to form aldehydes, which can then form imine bonds with unreacted amines.² Here we propose harnessing the endogenous LOX signals in post-VML skeletal muscle to confer improved mechanical and adhesive properties to gelatine-based hydrogel implants for muscle defects.

METHODS: Gelatine methacryloyl (GelMA) was synthesised according to methodology described by Loessner *et al.*³ The degree of modification of the GelMA was characterised using ¹H-NMR spectroscopy. Hydrogel crosslinking kinetics were characterised by rheology. LOX-induced stiffening experiments were conducted by swelling the GelMA in PBS or PBS containing 0.5U/ml LOX. LOX-induced stiffening of hydrogels was characterised by imine bond formation using FTIR.

RESULTS: GelMA (DoM = 82%, 20wt%, UV-crosslinked for 5 mins) had a preswollen compressive modulus of 140kPa. After swelling for 2 days in PBS/(-)LOX, this reduced to 50kPa. After 2 days of swelling in PBS/(+)LOX, the compressive modulus was 150kPa. FTIR showed imine bond formation in the PBS/(+)LOX-swollen hydrogels, but not in the PBS/(-)LOX-swollen hydrogels.

DISCUSSION & CONCLUSIONS: Mechanical characterisation showed that UV-crosslinked GelMA hydrogels incubated in PBS/(+)LOX regained their pre-swollen mechanical properties, whereas hydrogels incubated in PBS/(-)LOX did not. FTIR confirmed that this was due to the formation of imine bonds within GelMA hydrogel in the presence of LOX.

These preliminary results show that GelMA scaffolds are suitable substrates for LOX-mediated stiffening, confirming that these hydrogels are enzyme-responsive. The next steps involve confirmation of LOX-responsive tissue adhesion of the GelMA hydrogels. The adhesive properties can then be further optimised by the addition of chemical motifs to stabilise the bonds to tissue.

ACKNOWLEDGEMENTS:

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Keywords: Hydrogels and injectable systems, Polymers - natural / synthetic / responsive

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Porous poly(glycerol sebacate) methacrylate nerve guidance conduits for peripheral nerve regeneration

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INTRODUCTION: Following peripheral nerve injury, nerve guidance conduits (NGCs) are implanted to bridge gaps between nerve stumps as an alternative to nerve autograft. Conventional NGCs are simple tubes, usually stiffer than the native tissue, and unable to facilitate regeneration over larger gaps. Poly(glycerol sebacate)-methacrylate (PGSm) is a synthetic UV-curable biodegradable material with favourable mechanical properties. The aim of this study was to assess the biocompatibility of novel PGSm conduits in vitro.

METHODS: PGS prepolymer was synthesised via a polycondensation reaction between glycerol and sebacic acid and functionalised via the addition of methacrylation moieties prior to NMR analysis. Polymerised high internal phase emulsions (PolyHIPEs) were created and injected into a silicone mould before UV-curing to produce porous PGSm conduits. Scanning electron microscopy (SEM) was used to examine scaffold morphology.

NG108-15 neuronal cells were seeded in serum-supplemented medium onto bilaterally dissected non-porous and porous PGSm conduits and cell proliferation measured using resazurin assays. In other experiments, the seeded scaffolds were treated with serum-free medium after 48 hours to induce neuronal differentiation, which was determined by immunolabelling for β III-tubulin, confocal microscopy and image analysis to measure neurite length; this was compared to neuronal differentiation on standard tissue culture plastics. Cell viability was determined using Calcein AM and ethidium bromide (LIVE/DEAD) staining.

All experiments were performed at least 3 times and one-way or two-way ANOVA tests with multiple comparisons were used for statistical analysis.

RESULTS: NMR peaks confirmed the chemical composition of sebacic acid, glycerol and methylene groups. SEM of the PGSm scaffolds demonstrated the presence of highly interconnected pores with an average pore size of $7.38\mu\text{m}$ ($\pm 2.26\mu\text{m}$). In NG108-15 cell-seeded PGSm scaffolds, cell proliferation increased significantly over a 7-day period on porous and nonporous scaffolds, whilst LIVE/DEAD staining showed high cell viabilities, which were not significantly different to TCP controls. Immunocytochemistry showed that average and maximum NG108-15 neurite lengths on PGSm conduits were not significantly different to those on TCP controls.

DISCUSSION & CONCLUSIONS: Porous PGSm conduits are necessary to allow diffusion of nutrients and growth factors between the extraneural environment and intraluminal space within the conduits. The pores also help facilitate better axonal regeneration, while pore sizes of $<10\mu\text{m}$ inhibit migration of scar tissue-forming fibroblasts that inhibit such regeneration. This study has shown the biocompatibility of porous PGSm conduits to neural cells in vitro and supports their further exploration for the optimised growth of primary neural cells in ex vivo and in vivo studies.

Keywords: Biomaterials, Nervous system (brain-central-peripheral / disorders)

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Endogenous Protease Mediated Release Of Engineered Extracellular Vesicles

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INTRODUCTION: Chronic and aberrant inflammation negatively impacts tissue repair. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) possess background properties that are beneficial to tissue repair. The objectives of this study were: 1) To engineer MSC-EVs with enhanced anti-inflammatory properties. 2) Develop an alginate-based EV release system with an MMP2 enzymatic fusion-peptide for release of the engineered EVs during the inflammatory phase of tissue repair.

METHODS: We engineered MSCs to generate EVs with increased levels of miR-22-3p using a lentiviral system to target the NLRP3 inflammasome pathway. Inflamed macrophages were treated with the engineered EVs or controls, and inflammatory marker expression/NLRP3 pathway targets were quantified using qRT-PCR, immunoblotting and ELISA. The MMP2-RGD fusion peptide was conjugated to alginate backbone as per published protocols. MMP2-mediated release of the peptide from the backbone was quantitatively evaluated by BCA assay. EV release from hydrogels (control and experimental groups) in the presence of MMP2 was evaluated using quantitative fluorescence-based assay (n=6). Anti-inflammatory function of hydrogel-released EVs on macrophages was measured using qRT-PCR and ELISA (n=4). Student's t-test or ANOVA followed by Tukey's ad hoc test were used for statistics (CI=95%).

RESULTS: miR-22-3p expression was enhanced in engineered MSC-EVs compared to controls. miR-22-3p engineered MSC-EVs had enhanced anti-inflammatory activity compared to naïve MSC-EVs. MMP2 cleaved the fusion peptide and released the peptide into solution compared to RGD alone controls. The quantity of EVs released from the hydrogel increased in the presence of MMP2. Engineered MSC-EVs released from the hydrogel retained their anti-inflammatory function when evaluated on primary mouse macrophages.

DISCUSSION & CONCLUSIONS: MSC-EVs can be engineered to present miR-22-3p to target cells for enhanced anti-inflammatory function. Alginate hydrogels carrying a fusion MMP2 enzymatic domain and RGD (EV-binding) domain can be used to release functional EVs based on MMP2 activity. As MMP2 is present during inflammation and macrophage recruitment, this delivery system will allow for temporally targeted delivery of engineered anti-inflammatory EVs at the wound site.

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Keywords: Multipotent (mesenchymal) stem cells, Wound healing

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Porous Poly(glycerol sebacate)-methacrylate Scaffolds For Adipose Tissue Engineering

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INTRODUCTION: Autologous fat grafting is commonly performed during reconstructive surgeries to repair soft tissues. However, this transplanted fat has poor long-term survival and often additional procedures are required to achieve the desired tissue volume, increasing costs and patient trauma¹. As such, there is a need for an improved fat graft that can maintain volume following transplantation. Adipose tissue engineering aims to develop biomaterials that facilitate the survival and revascularization of transplanted adipose tissue, promoting long term tissue volume post-transplant. Poly(glycerol sebacate) (PGS) is a synthetic biomaterial which is highly suited to adipose tissue engineering, as it is much softer than commonly used biomaterials and is also highly elastomeric², mimicking the mechanical properties of adipose tissue. We herein report the development and optimisation of porous PGS-methacrylate (PGS-M) scaffolds for adipose tissue engineering applications.

METHODS: Polymerised high internal phase emulsion (polyHIPE) templating of photocurable PGS-M pre-polymer was used to create porous three-dimensional scaffolds. The parameters of scaffold fabrication were altered to investigate the effect on pore structure. Scaffolds were characterised using scanning electron microscopy (SEM) and the mechanical properties of PGS-M scaffolds were measured. PGS-M scaffolds were seeded with adipose-derived stem cells (ADSCs) to assess cell metabolic activity and migration.

RESULTS: PGS-M was synthesised and found to have a degree of methacrylation (DM) within an acceptable range of the theoretical DM. Scaffolds were fabricated with a range of pore sizes (varying from microporous to macroporous) and interconnectivity depending on HIPE parameters. PGS-M scaffolds showed elastomeric properties, with mechanical properties within the range of adipose tissue. The metabolic activity of ADSCs cultured on PGS-M scaffolds increased over the time course of the experiment, indicating that the cells were proliferating on the scaffolds. Migration of ADSCs into the scaffold could be observed following 14 days of in vitro culture.

DISCUSSION & CONCLUSIONS: These results indicate that PGS-M polyHIPE scaffolds are suitable for the culture of ADSCs, and by altering the internal phase of the emulsion, the pore size can be increased, promoting cell migration through the scaffold. Ongoing studies will focus on assessing the angiogenic potential of these scaffolds in order to develop viable adipose tissue constructs.

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Keywords: Polymers - natural / synthetic / responsive, Biomaterials

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Biomimetic mineralization of novel graphene oxide functionalized gelatin methacryloyl microgel for in situ bone defect repair

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INTRODUCTION: Large bone defects caused by trauma, infection, and tumor are common clinical problems. The current clinical treatments focus on the application of autogenous grafts, allografts, and xenografts despite several inherent deficiencies, including donor site damage, potential immune rejection, disease transition, and high costs. Therefore, developing an ideal bone graft substitute has important clinical value. Gelatin methacryloyl (GelMA), a photo-crosslinkable polymer, has been widely applied in bone tissue engineering due to its excellent biocompatibility and cell-responsive properties. While the low mechanical strength and poor osteoconductivity limit its further application. Graphene oxide (GO), a two-dimensional nanomaterial with abundant functional groups and superior mechanical strength, has attracted plenty of attention as an inorganic additive in biopolymers for constructing novel composite biomaterials. Hydroxyapatite (HAp), the main component of bone, has elicited enormous interest in the domain of bone tissue engineering owing to its excellent biocompatibility and osteoconductive potential. Based on this observation, we hypothesized that developing GO-functionalized GelMA hydrogel with a HA coating could be a potential strategy for accelerating the healing of large bone defects.

METHODS: In this study, the GO-GelMA-HAp microgel, with a size of 1500µm, was constructed by molding in microarray chip, cross-linking under 405nm UV light, and biomimetic mineralizing in simulated body fluid (SBF). Systematic in vitro experiments were conducted to evaluate the physiochemical properties of the microgel and to estimate the cell viability and cell proliferation of the microgel. Moreover, in vivo experiments were performed to evaluate its ability to repair rat calvarial bone defects.

RESULTS: In this study, the resulting GO-GelMA-HAp microgel exhibited a porous and interconnected structure coated with HAp crystal on the surface. The elastic modulus of the GO-GelMA-HAp microgel was higher than GelMA microgel. Furthermore, the GO-GelMA-HAp microgel showed higher cell adhesion and proliferation in vitro and higher bone formation in vivo.

DISCUSSION & CONCLUSIONS: The incorporated GO enhanced the mechanical strength and provided additional functional groups for GelMA hydrogels. These functional groups provided additional bioactive sites for HA deposition on the one hand and adsorbed the surrounding cells and proteins on the other hand. This study provides new insights for developing bone substitutes for bone tissue engineering.

Keywords: Biomaterials, Composite materials

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Graphene Oxide-coated Titanium Alloys for Bone Tissue Regeneration

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INTRODUCTION: Bone defects over a critical size will not spontaneously heal. This is a major clinical problem as damage to bones is extremely debilitating and common[1]. Joint arthroplasties are also increasingly common due to our aging population. For surgical interventions to work when treating these problems the materials used are of crucial importance. Titanium (Ti) is used widely in orthopaedic surgery for replacing bone. While it has good properties generally, it is bioinert meaning it will not interact with a biological system and will not encourage new bone growth. Ti could be enhanced with graphene oxide (GO)[2,3].

METHODS: GO was deposited onto Ti64 (surgical alloy) via electrodeposition for varying lengths of time. The coated material was analysed by SEM and Raman spectroscopy. Wettability was also assessed by contact angle measurement and the roughness of the surfaces by white light interferometry. Human osteoblasts were cultured on the materials and a LIVE/DEAD assay performed in order to assess cell viability and cell behaviour and morphology. Viability and cell numbers have also been investigated with alamar-blue and pico-green assays respectively.

RESULTS: Raman spectroscopy confirmed the presence of GO on the Ti surface and that it was fairly evenly spread after the deposition process. The wettability of the materials saw a gradual increase after longer GO deposition times. Roughness of the materials showed some statistically significant differences but were all well below the upper limit of "smooth" perception for osteoblasts, implying the materials' roughness may be discounted as a factor in cell response. Osteoblasts showed enhanced viability and more pronounced fibroblastic morphology (implying the cells were spreading) when cultured on materials coated in GO compared with the uncoated material.

DISCUSSION & CONCLUSIONS: If bone regeneration can be made more rapid then a large portion of the socioeconomic burden can be lifted. GO provides a promising platform for enhancing current materials with a large scope for more advanced modifications such as the functionalisation of the material with active biomolecules. GO is biocompatible and easily produced meaning it is suitable for scaling up and use in healthcare, we have shown that it can easily be deposited on Ti and scaling up of this process is also very achievable.

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Keywords: Biomaterials, Nanomaterials (inc graphene)

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Fabrication and Characterisation of Monomeric Type I Collagen Scaffolds as Tissue Engineering Heart Valve

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INTRODUCTION: With the increasing number of global cardiovascular disease cases and the limitation of clinically used traditional mechanical and bioprosthetic valves, natural polymer tissue engineering heart valve (TEHV) has been reckoned as a new candidate in valve replacement. Among many synthetic and natural polymers, type I collagen (COL1) is widely investigated for its excellent biocompatibility, biodegradability, and structural resemblance to the extracellular matrix. Although most research has been focused on the use of polymeric collagen, this might give rise to material antigenicity. Therefore, this study is set to investigate the possibility of constructing a monomeric COL1 scaffold design with desired microstructure and mechanical properties to serve as TEHV.

METHODS: Monomeric COL1 experienced fibrillogenesis before being crosslinked using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS), or directly cast into polytetrafluoroethylene (PTFE) moulds, which were then freeze-dried to yield porous scaffolds.

Atomic force microscopy (AFM) was employed to examine fibrillar reconstitution of collagen suspension pre- and post- fibrillogenesis. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were involved in scaffold microstructure and chemical structure characterisation. Compressive, tensile, and viscoelastic property information of dry scaffolds was provided by testing scaffolds using dynamic mechanical analysis (DMA), while bending moduli in hydrated state were obtained by conducting self-deflection test on wet samples. The viscosity and viscoelasticity of collagen suspension before moulding were also investigated.

RESULTS: Monomeric COL1 was shown to possess the ability to form reconstituted fibres after fibrillogenesis. Both fabrication and crosslinking methods were proved to successfully produce porous scaffolds with desired porosity and suitable pore size without damaging collagen chemical structure. Crosslinking was confirmed effective by both increased amide and ester band intensity and higher stiffness of scaffolds (tensile modulus for bovine skin-originated COL1 improved from 1.13MPa to up to 2.21MPa; and for crosslinked bovine amnion-originated COL1, tensile modulus reached 4.06MPa). Hydrated bending modulus is within desired theoretical range for cell activities. Viscosity data is proposed to relate to the final pore size formed.

DISCUSSION & CONCLUSIONS: In conclusion, the monomeric COL1 scaffolds produced exhibited promising properties towards the application of alternative heart valve replacement from the mechanical perspective. Meanwhile, the scaffolds were structurally and mechanically suitable for further in vitro and in vivo work.

Keywords: Biomaterials, Cardiovascular

PP-274

Determination of changes in the physicochemical properties of dressing biomaterials that lead to different protein adsorption

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INTRODUCTION: Diabetic foot and leg ulcers as well as decubitus ulcers affect millions of individuals worldwide leading to poor quality of life, pain and in a number of cases to limb amputations. Despite the global dimension of this clinical problem, limited progress has been made in developing wound dressings able to promote healing. The aim of this study was to determine if changes in the physicochemical properties of dressing biomaterials could lead to different protein adsorption patterns relevant to tissue regeneration

METHODS: The swelling, morphology and protein adsorption of commercially available wound dressing were analysed. The dressings considered were: Melolin (a polyester-based product), Kaltostat (a agarose based product) and Aquacel and Keramax (methylcellulose based biomaterials). The swelling kinetics of each dressing were assessed by gravimetric analysis over 24 hours in two groups incubated either in Radin's simulated body fluid (SBF) or serum. After this period, scanning electron microscopy (SEM) of the dry control dressings as well as environmental scanning electron microscopy (ESEM) of the wet samples were performed to study the polymer fibre morphology upon swelling. Protein adsorption was determined by incubating the disc in serum for 1h and 24 h. Excess serum was washed off in copious volumes of deionized water and the samples were finally incubated in Laemmli's buffer for Sodium dodecyl sulfate Poly(acrylamide) Gel Electrophoresis (SDS-PAGE) to elute the protein bound to the surface of the polymers. The eluted protein samples were finally characterized by 10% SDS-PAGE and stained by silver staining (Biorad, UK) Swelling data were statistical analysed by the Analysis of Variance (ANOVA).

RESULTS: The swelling for different wound dressing groups indicated that the cellulose-based wound dressings (Keramax, Aquacel) showed the highest absorption rates with significant differences between different groups. SEM and ESEM showed flat fibres in the case of Kaltostat, while the others showed compact fibres with a round cross section. SDS-PAGE showed that albumin was the dominant adsorbing protein in all the wound dressing groups suggesting a passivation of their surface not conducive of cell colonization (Fig.1).

DISCUSSION & CONCLUSIONS: The present work indicates that, while able to absorb high liquid volumes, the analysed dressings expose a surface mainly conditioned by an albumin layer that can prevent cell adhesion and hence not able to promote tissue regeneration within the wound bed.

Keywords: Biomaterials, Wound healing

PP-275

Spectroscopic modalities for detection of biomaterial degradation through macrophage activation

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INTRODUCTION: Biodegradable biomaterials have wide applications as temporal scaffolds or drug carrier. These implanted materials as foreign substance to the body will induce immune response. Macrophages are professional phagocytes for ingesting and eliminating foreign particles. The phagocytosis process internalizes degradable materials particles through forming specialized vacuole, phagosome, and maturing the phagosome to phagolysosomes which contain various acidic enzymes. The harsh digesting environment within macrophages will add extra factors to degrade biomaterials. In this project, we aim to assess whether the spectroscopic modalities can detect and follow the degradation process of degradable biomaterials, in particular, poly(glycolic acid) (PGA) within macrophages and the associated influence factors.

METHODS: The RAW 264 cell line was used as the macrophages' model. Two forms of PGA, one in fiber form, and another in porous foam were used. Both PGA materials were grounded into $\leq 5\mu\text{m}$ particles for macrophage internalization at density of 20, 10, 5 particles/cell. The RAW cells were polarized from M0 to M1 phenotype by LPS and IFN- γ before adding PGA particles for internalization. The visible and fluorescence images have been taken at culture day 0, 2 and 4. The RAW cells with and without internalized PGA particle groups were collected and cytopun to microscopic glass coverslip after these culture times, and fixed by paraformaldehyde for FTIR analysis. The crystallinity of PGA particles before and after internalization was analyzed by a DSC. NO and other cytokines released from the RAW cells were analyzed.

RESULTS: FTIR spectra demonstrated characteristic spectra between RAW cells with and without internalization of PGA particles. The internalization process was fast. There were limit spectral changes for control RAW cells (no PGA internalization) during the 24 hour culture. There were notable spectral changes for RAW cells with internalized particles in comparison to control cell group and the PGA forms dependent. The PGA internalization added new PGA peaks in fingerprint and lipid regions with subtle spectral change to the pure PGA spectra. The PGA internalization triggered notable spectral changes in amine I, II, III and lipid regions. Fibrous PGA triggered more immuneresonse than foam PGA to RAW cells by NO and cytokine analysis.

DISCUSSION & CONCLUSIONS: Spectroscopic modalities, in particular FTIR spectroscopy, can be a powerful tool to study degradation process of biomaterials through intervention of macrophage activation dynamically and spatially. The label-free features of the modalities will reduce artifacts incurred in the destructive modalities.

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Keywords: Enabling technologies, Immunity / immunomodulation / macrophage

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Insight on the antibacterial potential of Cu-doped sol-gel coatings: a proteomic approach

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INTRODUCTION: The development of new biomaterials with antibacterial properties is crucial to prevent infections. Cu, with antimicrobial and osteogenic functions, supposes an interesting option to bioactivate prostheses [1]. However, the bactericide mechanism of Cu-derived materials remains poorly understood [2]. Therefore, this study aims to evaluate the interaction between Cu-materials and biological systems. For that, sol-gel coatings with increasing amounts of CuCl₂ were developed and physicochemically characterized. Their biological properties were evaluated *in vitro* and their interaction with serum proteins analyzed using proteomics. ²⁺

METHODS: The materials were synthesized using methyltrimethoxysilane and tetraethyl orthosilicate through the sol-gel route. 0.1, 0.5, 1 and 3 %wt CuCl₂ were added to the sol-gel mixtures. These compositions were applied as coatings onto Ti discs. The physicochemical characterization was carried out using SEM, EDX, FT-IR, NMR and XRD, and the Cu²⁺ release kinetics measured through ICP-AES. *In vitro* studies with both HOb and THP-1 were performed to evaluate the osteogenic and inflammatory potentials of the coatings, respectively. Their antibacterial activity was determined using *E. coli* and *S. aureus*. The effect of Cu ions on the adsorption of human serum proteins onto the material surface was evaluated through nLC-MS/MS.

RESULTS: The Cu-doped coatings were homogeneous. Cu²⁺ ions were detected in the coatings through EDX and XRD. The coatings were no cytotoxic. Cu increased the expression of BMP-2, ALP and OCN in HOb. Moreover, a higher expression of INF- δ , TNF- α and IL-1 β and an elevated secretion of TNF- α were measured with THP-1 cultured with the Cu-doped coatings. The coatings showed a complete inhibition in the viability of *E. coli* and *S. aureus* for materials with 1 and 3 %wt CuCl₂. Proteomic analyses showed that Cu-doping increased the adsorption of proteins related to immunity (CO5, C4BPA, C4BPB, CO8B, SAMP, ENOA).

DISCUSSION & CONCLUSIONS: Cu-doped sol-gel coatings showed osteogenic and antibacterial properties, and increased inflammatory potential. This fact correlated with the higher adsorption of proteins related to the complement system activation. This pathway activates macrophage recruitment and lead to membrane attack complexes formation, promoting the coating antibacterial effect. Therefore, our results deepen the knowledge about the antibacterial mechanisms associated with Cu-enriched biomaterials.

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Keywords: Omics / bioinformatics and systems biology, Infection

PP-277

Evaluation of the physical, mechanical and biological properties of a novel biomedical hydrogel: implication for gingival-based and oral healthcare applications

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INTRODUCTION: There is a demand for an accurate representation of human gingival tissue for both clinical and biomedical applications. Primarily, this requires the selection of a substrate that mimics the native extracellular matrix which also maintains the correct substrate characteristics (i.e. lamina propria) and favor growth of the fibroblasts and epithelial cells. The aim of study was to develop and evaluate novel hydrogels that could potentially be used for a 3D, human-based, gingival model.

METHODS: Rat tail collagen, 10% gelatin methacryloyl (GelMA), and 3.5% or 2.5% sodium alginate (SAH, SAL, respectively) were investigated. GelMA samples were photo-crosslinked using 0.3%wt lithium phenyl-2,4,6-trimethylbenzoylphosphinate, and UV exposure for 60 s; whereas SAH and SAL samples were crosslinked by CaCl₂ to form hydrogels. Rat tail collagen was incubated overnight for gelation. In addition, composite formulations of GelMA-SAH and GelMA-SAL were crosslinked using either UV-only, CaCl₂-only, UV-CaCl₂, or CaCl₂-UV to ascertain the role of sequential crosslinking. All hydrogels were characterized using SEM, FT-IR, WCA, DSC, rheology and their in vitro biocompatibility assessed using human primary gingival fibroblast cells. DMA was also used to compare the profiles of the hydrogels against native animal gingival tissue.

RESULTS: Microscopy summarised noticeable differences between gels. FT-IR profiling was able to resolve changes in peak intensities corresponding to intermolecular interactions between SA and GelMA. WCA confirmed the hydrophobic nature of GelMA, and GelMA-SA hydrogels when crosslinked by UV or UV-CaCl₂. DSC and rheological investigation showed high melting points, viscosity, and viscoelastic values of GelMA-SA hydrogel when crosslinked by CaCl₂, or CaCl₂-UV. DMA-based mechanical characterization reported similarities to the storage modulus, loss modulus, and stiffness values of GelMA-SA when crosslinked with UVCaCl₂ compared with the animal gingival tissue. No determinantal effects in terms of biocompatibility were noticed with the samples.

DISCUSSION & CONCLUSIONS: In short, GelMA-SAH and GelMA-SAL hydrogels displayed superior properties compared with the other hydrogel samples. Moreover, the crosslinking methods offer a potential role on fine-tuning the mechanical properties of these hydrogels and could be tailored towards the properties of native animal gingival tissue.

Keywords: Biomaterials,

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3D Printing of Fibrous Biomaterials with Controlled Architectures for Peripheral Nerve Repair

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INTRODUCTION: Peripheral nerve injury affects over one million people each year worldwide [1]. Autografts are considered as the gold standard for nerve repair, but nerve guidance conduits (NGCs) remain the preferred alternative for small gap repairs. The development of novel biomaterials combined with 3D printing offers the potential for high accuracy and complexity and has gained significant interest in the field of nerve tissue regeneration [2]. Native extracellular matrix (ECM) derived biomaterials are among these promising new materials due to their high biocompatibility and ability to provide a biologically relevant microenvironment for cell culture [3]. However, ECMs often require exogenous crosslinking to provide adequate mechanical properties. In this work methacrylation of nerve ECM (nECM-MA) was chosen for its ease of use and control over crosslinking [4]. The overall goal of this study was to develop and characterise a new nerve-derived ECM tissue ink to build the next generation of NGCs using 3D printing.

METHODS: Nerve tissue was harvested from adult porcine hind legs. Nerve ECM material was obtained by pepsin solubilisation followed by methacrylation to obtain the final nECM-MA tissue ink. Photo-crosslinking was performed using a 405nm blue light with different light exposures (2-60 seconds). *In vitro* cell culture analysis using PC12 cells was performed to assess cell viability and neurite outgrowth compared to collagen and tissue culture plastic controls. Higher concentrations (2/4/8% w/v) of the ink were used to 3D print scaffolds and analyse the induced collagen fibre alignment.

RESULTS: Altering temporal light exposure was shown to modulate the stiffness of the ink and reach native tissue levels. Following seeding on 2D coatings, nECM-MA displayed better neurite outgrowth than the collagen control. With 3D printing, control over the alignment and thickness of the collagen fibres was achieved depending on the pH and speed of printing.

DISCUSSION & CONCLUSIONS: This work highlights the potential of a novel tissue ink to create next generation NGCs with tailored mechanical properties, improved neurite outgrowth and controlled architectures. Combining this tissue ink with 3D printing offers the potential to print NGCs with high accuracy and complexity with improved mechanical properties and enhanced cell behaviour driven by this aligned architecture mimicking that of native nerve tissue.

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Keywords: Nervous system (brain-central-peripheral / disorders), 3D printing and bioprinting

PP-279

Biocompatible collagen-based scaffolds loaded with mesenchymal stem cells for the regeneration of ligament to bone enthesis

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INTRODUCTION: The enthesis, or interface, between bone and ligament, has a highly sophisticated biomaterial function. Injury to the enthesis often requires surgical intervention, however, due to poor regeneration during healing, this transitional tissue is prone to rupture recurrence. Tissue engineering offers the potential to recover the functional integrity of enthesis. This study focused on developing collagen-based scaffolds for ligament-bone regeneration.

METHODS: Three collagen constructs were investigated to mimic the various regions: a soft tissue layer composed of type I monomeric collagen; an uncalcified fibrocartilage layer composed of type II monomeric collagen and chondroitin sulphate; and a calcified layer composed of type I monomeric collagen and hydroxy apatite.

In vitro studies were carried out to assess the biocompatibility and mesenchymal stem cell-adhesion ability of those scaffolds as a preparatory step for the future assessment of differentiation ability. Murine mesenchymal stem cells were isolated from bone marrow.

RESULTS: The isolated cells were proven to be negative for CD45 and CD11b and positive for CD44, CD105, CD73 and CD90 which is in agreement with literature as a confirmation of mesenchymal stem cells. All tested scaffolds were proven to be biocompatible with mesenchymal stem cells as indicated by a viability of above 80% after 6 weeks of culture. Mesenchymal stem cell adhesion and propagation on the surface of all tested scaffolds were also proven.

DISCUSSION & CONCLUSIONS: Together, the presented data confirmed the biocompatibility and adhesion ability of the prepared collagen-based scaffolds and paved the road for the assessment of cell differentiation ability and whether the compositional variations between collagen-based scaffolds have a significant effect on this ability.

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Keywords: Biomaterials, Interfaces - biological

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Customisation of GelMA hydrogel fabrication for muscle repair and regeneration

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INTRODUCTION: Muscles play a vital role in multiple dynamic events such as locomotion, mastication, as well as regulation of body metabolism. In the case where a volumetric muscle loss occurs, the body fails to restore and/or replace the lost tissue. As such, several novel approaches have been considered- such as reconstructive surgery and the use of tissue engineered scaffold- to aid the restoration of lost tissue. Recently, there has been increasing interest in the use of gelatin methacrylate, GelMA, which demonstrates outstanding biological properties owing to the presence of the arginine-glycine-aspartic acid (RGD) motif which facilitates cells to attach and proliferate. The unique reaction of gelatin and methacrylic anhydride (MA) allows GelMA hydrogels to be “customisable” photo-crosslinked to generate novel GelMA materials that exhibit improved and tunable mechanical properties compared to gelatin alone. In this work, the synthesis parameters of GelMA such as the quantity of MA used, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) concentrations, duration of UV exposure, and total reaction time were investigated and optimised for the specific application of muscle repair and regeneration.

METHODS: A number of techniques were utilized to evaluate the mechanical properties of these hydrogels and include ¹H NMR to determine the degree of substitution (DS), compression testing, rheology, swelling ratio, and a degradation assessment. For biological and biocompatibility characterization, the C2C12 muscle cell line was cultured within the GelMA hydrogels and evaluated by means of cell viability, cytotoxicity using AlamarBlue™, lactate dehydrogenase (LDH) release and Live/Dead staining, respectively.

RESULTS: The results indicated that increasing the MA content to 5% (v/v) resulted in higher degree of substitution and significantly lower cell viability compared to the 1% sample. Furthermore, increasing GelMA concentration to 20% (w/v) as well as increasing the UV exposure to 2 minutes resulted in enhanced mechanical properties i.e. a stronger hydrogel. LAP photoinitiator concentration of 0.1% (w/v) displayed highest cell viability and gel stability compared to 0.5 and 1%, while an increase in GelMA concentration to 20% resulted in decrease in cell viability by 50%.

DISCUSSION & CONCLUSIONS: Manipulating the synthesis parameters such as GelMA concentration and UV duration strongly affected the mechanical properties. While other parameters such as GelMA and LAP concentrations, UV exposure time, and MA content had a direct influence on the cell viability. Moreover, LAP concentration of 0.5% and above had negative influence on the stability of the hydrogels. Finally, the degree of substitution of GelMA increased as the reaction time and MA content increased.

Keywords: Hydrogels and injectable systems, Musculoskeletal (inc ligament / tendon / muscle / etc)

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Decellularized Human Adipose Tissue as an Alternative Graft Material for Bone Regeneration

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INTRODUCTION: Tissue engineering approaches to treat damaged bone include various tissue transplants such as autologous, allogeneic, and xenografts. Artificial materials have been widely introduced to meet the demand for graft materials, but insufficiency in supply is still not resolved. In this study, human adipose tissue, easily obtained from the human body, was harvested, and the tissue was decellularized to fabricate a decellularized human adipose tissue matrix (DM) as an alternative graft material.

METHODS: Human adipose tissue was obtained via liposuction. The obtained fresh adipose tissue sample was cut into pieces then put into decellularization solution (1% antibiotic-antimycotic solution and 1% phenylmethanesulphonyl fluoride). Lipids were further removed via treatment in isopropanol. The sample was then subjected to another enzymatic digestion and lipid removal processes. The obtained decellularized adipose tissue matrix was lyophilized to form a graft material in disc shape.

RESULTS: Decellularization was confirmed by nuclear staining methods and detection of RNA and DNA via PCR. Bone morphogenetic protein 2 (BMP2)-loaded DM showed the ability to form new bone tissue when implanted in subcutaneous tissue. In recovery of a mouse calvarial defect model, BMP2-loaded DM exhibited similar levels of bone tissue regeneration efficiency compared with a well-defined commercial product, BMP2-loaded CollaCote®.

DISCUSSION & CONCLUSIONS: The DM developed in this study is expected to address the problem of insufficient supply of graft materials and contribute to the treatment of bone defects of critical size as an alternative bone graft material with preserved extracellular matrix components.

Keywords: Decellularised matrices, Bone and bone disorders (osteoporosis etc)

PP-282

The synthesis, development and enhancement of a P(3HB-co-4HB)-bioactive glass-graphene composite for biomedical engineering applications

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INTRODUCTION: Wound management has been a critical challenge. Naturally derived polymers have always been the subject of interest, especially microbial-derived materials as they offer the desirable biocompatible and biodegradable characteristics in addition to negligible contamination threat and flexibility in processing. Sol-gel derived bioactive glass (BG) is a well-documented osteoinductive material for hard tissue engineering. BG has also been found to support soft tissue regeneration via the dissolution of ions as well as promoting cell proliferation, differentiation, and angiogenesis. In addition, liquid exfoliated graphene demonstrates a degree of antimicrobial activity due to its magnetic properties as well as its pro-angiogenic/vascularisation behaviour. Taken together, these characteristics would contribute to an ideal wound dressing material and, as such, this project focuses on the use of P(3HB-co-4HB) copolymer, derived from Cupriavidus Necator USMAA1020, to develop a novel BG-graphene composite wound dressing.

METHODS: The composite was homogenously combined via facile blending before being fabricated via solvent casting. Physical and mechanical characterisation of the composite was achieved using SEM, FTIR, DMA, DSC and WCA. Biocompatibility was assessed using dermal human primary cells (HDF) and a commercial-available fibroblast cell line (BJ, CRL-2522) and standard cell culture assays including cell metabolic activity (MTS), LDH-release, viability (AlamarBlue) and the Live-Dead staining assays. Protein expression was studied via human inflammation antibody cytokine array and preliminary animal study as to further study the biocompatibility and immunological response of the composite in promoting wound healing.

RESULTS: The BG-graphene incorporated P(3HB-co-4HB) copolymer composite demonstrated enhanced physical and mechanical characteristics by means of DMA and DSC analyses compared to the polymer only sample. Moreover, the hydrophilicity of the composite was significantly higher ($p < 0.05$) which is known to facilitate cells adhesion on a material. Enhanced attachment, viability and proliferation of dermal cells was observed following culture on the composite. A corresponding increase in immune cell infiltration, adhesion alongside the subsequent production of inflammatory cytokines was observed. Antimicrobial activity study showed a positive result against microbial colonisation. CAM assessment demonstrated good biocompatibility alongside an enhancement of angiogenesis. This was further corroborated with the in vivo studies which summarised well-integrated bioactivity characteristics, revascularisation of the local microenvironment and an effective immune response, alongside positive wound closure between the composite and the native tissues.

DISCUSSION & CONCLUSIONS: Taken together, this study highlights the sustainable development of a natural bioactive polymeric carrier that promotes the wound healing process- potentially addressing

patient compliance over wound management and improving quality of life.

Keywords: Biomaterials, Wound healing

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Evaluation of Mucilage as a Plant Secret for the Production of Bioactive Tissue Engineered Scaffolds

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INTRODUCTION: The physical properties (porosity, high water holding capacity, mechanical stability, flexibility, controlled degradation behavior) that are aimed to be gained by preserving the biological basis (biocompatibility, antimicrobial activity, wound healing) of scaffolds to be obtained by processing plant mucilages, and these three-dimensional structures have a high ratio to the natural ECM structure. expected to approach. Within the scope of the study, polysaccharide-based mucilages to be obtained from flax seed (FS) were evaluated in the cryogels production processes. Natural and reliable new biomaterials that have the potential to be used in tissue engineering applications will be produced by evaluating such natural resources, which are traditionally used for many years and defined as medicinal plants, in the light of scientific data.

METHODS: To obtain FS mucilage (FSM), seeds were mixed with distilled water at a 1:25 weight ratio overnight at 80°C for the gel to pass into the water. The mucilage was filtered with a multi-layered gauze strainer to separate it from the seed residues. The resulting viscous gel was dried using a freeze drier. To produce 3D gel structures, solutions of the obtained dry mucilages in distilled water at different concentrations (wt%) were prepared. The 5 mL of each solution was mixed with a crosslinking agent and poured immediately into plastic syringes and kept in the freezer at -20°C for 24 hours for crosslinking. The frozen gels in the molds were then thawed, washed, and lyophilized to obtain the 3D porous cryogel structures. The chemical structure, morphology, contact angle measurements for surface properties, swelling ability, texture analysis for the mechanical properties, and degradability behavior of cryogels were studied. Antibacterial activity using disc diffusion, cytotoxicity with MTT assay, and cell morphology investigations by SEM analyzes were also performed.

RESULTS: The cryogels exhibited an interconnected porous structure. Gels with hydrophilic surfaces have the ability to absorb water up to an average of one hundred times their own weight. The gels showed antimicrobial activity against gram-positive and gram-negative bacterial strains. It was also observed that FSM did not show any toxic effect on cells.

DISCUSSION & CONCLUSIONS: A novel cryogels synthesis were carried out successfully by using a biologically based, cost-effective and very important natural resource that can mimic the ECM structure. Studies are going on to add functional properties to scaffolds by adding active ingredients to FSM cryogels.

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Keywords: Polymers - natural / synthetic / responsive, Biomaterials

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Use Of Peptide Hydrogels (PeptiGels®) As A Matrigel® Full Or Partial Replacement For Scaling Up Production Of Colorectal Cancer (CRC) Organoids

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INTRODUCTION: Organoids (tissue specific cell clusters) form reductionist models of total or partial organ function[1]. These models hold value in many sectors e.g. medical development. Colorectal cancer (CRC) organoids are of specific interest due to high patient morbidity from this cancer type, coupled with low drug trial successes[2,3]. Current gold-standard production relies on Matrigel® use; a murine sarcoma-based hydrogel[4]. Matrigel presents multiple drawbacks including inconsistency, ethical concerns, and supply issues[5]. Peptide hydrogels, such as PeptiGels®, present a promising fully synthetic alternative[6]. This study will assess PeptiGels® suitability for full or partial Matrigel replacement within established organoid growth processes.

METHODS: This study examined six PeptiGels® (α 1, α 2, α 2+RGD, α 4, γ 2, γ 4+RGD) suitability for CRC organoid growth with consideration of scale-up suitability. This included mechanical property consideration, dynamic mechanical analysis (DMA), and shear testing for use within dynamic environments. Organoid formation will also be tested for single cell and fragmented encapsulation, investigating viability over multiple passages. Imaging including staining alongside spent media analysis will assess these qualities.

RESULTS: DMA results showed decreases in Young's modulus (E) in all PeptiGels® (excluding α 4), with all showing sufficient E values to be viable for further consideration, with α 2 presenting the highest E and α 4 the lowest.

Further results will include comparison of growth rates, organoid viability, and consistency of formation for investigation of single-cell initial seeding, fragmented seeding, and viability for freeze-thaw procedures. Incorporation with Matrigel® and exploration of environmental effects on viability will be examined.

DISCUSSION & CONCLUSIONS: All tested PeptiGels® produced promising results for their ability to withstand necessary processes for organoid culture, as demonstrated through DMA analysis. Previously, PeptiGels® have shown shear properties suitable for bio-printing, demonstrating their suitability for structural formation beyond standard droplet culture in plates [7]. Future work will focus on selection of specific PeptiGels® for CRC organoids. In conclusion, this data supports the continued investigation of PeptiGels for full or partial Matrigel replacement for CRC organoid growth.

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Keywords: Hydrogels and injectable systems, Cancer

PP-285

Jellyfish collagen filled Titanium matrix for large bone defect repair

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INTRODUCTION: Large bone defects can be caused by trauma, disease, surgery or tumour resection where the bone is unable to heal as normal due to the size of the defect or fracture. To understand and resolve this clinical problem, regenerative medicine approaches, particularly tissue engineering and the use of biomaterial, have been widely studied and used. A recent type of collagen that has emerged is jellyfish collagen (Jellagen), which is collagen type 0, non-cytotoxic and biocompatible. Titanium (Ti) and its alloys are clinically established in bone implants. In this project, we are investigating whether a hybrid jellyfish collagen – Ti scaffold can provide a natural 3D microenvironment for bone formation.

METHODS: Ti (Ti-6Al-4V) matrices were infilled with jellyfish collagen and then freeze-dried to produce the scaffolds. Human osteoblasts were then seeded onto these for up to 21 days. AlamarBlue reagent and Live/Dead® staining were used to assess cell viability and proliferation. Immunostaining of a key bone marker was chosen to observe bone cell-scaffold interaction and mineralisation while scanning electron microscopy (SEM) was done to observe cell morphology and adhesion to the different parts of the scaffold.

RESULTS: The viability results showed that the hybrid scaffolds supported cell viability, further confirmed by the Live/Dead images also showing proliferating cells throughout the scaffolds. It was evident from the immunostaining and SEM that the bone cells grew well in the hybrid scaffold and adhered strongly to the Ti and collagen across 14 days, with cell-to-cell and cell-to-biomaterial interactions visible.

DISCUSSION & CONCLUSIONS: The promising results of the early experiments of this project have shown that the hybrid jellyfish collagen - Ti scaffold supports human osteoblasts very well and encourages growth. This suggests that the hybrid scaffold provides an ideal 3D microenvironment for the bone cells. One of the next steps would be to assess bone formation long-term, up to 28 days.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

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Bioresorbable Phosphate-based Glass Fibres for Wound Healing Applications

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INTRODUCTION: There is a great demand for biomaterials capable of stimulating healing and preventing infections to address the global unmet need for the treatment of acute and chronic wounds. Phosphate-based glasses (PG) in the system P_2O_5 -CaO- Na_2O have shown great potential to induce tissue regeneration, whilst being replaced by regenerated tissue and releasing antibacterial ions. Specifically, electrospun phosphate-based glass fibres (PGF) are of interest in this study due to their fibrous morphology, capable of producing wound dressing mats.

METHODS: Coacervate gels containing 0.5, 1, 3 and 5mol% silver oxide (Ag_2O) were synthesised via a coacervate method and electrospun at 16.5kV and 2ml.h⁻¹ flow rate to produce PGF. The resulting fibres were characterised using X-ray diffraction (XRD), Raman spectroscopy, Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy. The PGF ion release in de-ionised water at 10mg/10ml was measured at 3, 24, 48 and 72h using microwave plasma atomic emission spectroscopy. The antibacterial activity was assessed against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), incubated at 37°C for 24h with absorbance measured using a CLARIOStar plate reader. The in-vitro healing potential of PGF was assessed via a scratch assay model using human adult skin keratinocyte cells (HaCaT).

RESULTS: We successfully manufactured PGF via electrospinning of coacervate gels. This manufacturing method is cost-effective, water-based and sustainable, making it an attractive option for scaled-up manufacturing. XRD demonstrated that all PGF were amorphous at all silver contents. SEM imaging showed fibres with an average diameter of 4.5µm and several µm in length. Release studies demonstrated that with an increase in silver content the Ag^+ release increased, as expected. An increase in silver content also reduced the amount of phosphorous released, however no significant trends in calcium and sodium release were found. PGF showed antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, with activity increasing with silver content and was more effective against *S. aureus* than *E. coli*. PGF release products were effective in promoting wound closure. In particular, PGF containing 3 and 5mol% Ag_2O significantly increased the average % gap closure between HaCaT cells when studied using a scratch assay.

DISCUSSION & CONCLUSIONS: PGF in the system P_2O_5 -CaO- Na_2O with the addition of Ag_2O have been prepared via electrospinning of coacervate gels. This work demonstrated that PGF containing silver ions have a great potential for the promotion of wound healing and the prevention of wound infections.

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Keywords: Wound healing, Fibre technology

PP-287

Biomaterials Based on the Extracellular Matrix as the Future of Tissue Engineering. Is There an Upper Limit on the dECM Content in Bioprinting Tissue?

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INTRODUCTION: The key element of bioprinting is the use of appropriate biomaterials that ensure the viability and functionality of the cells used in this process. One of the most popular biomaterials are those based on the dECM. The aim of this study was to find answers to these questions: what is the most optimal dECM concentration in the bioink?

METHODS: Bioinks precursors were prepared in a enzymatic method. The final bioink contained from 2%-16% dECM. Constructs were use for the assessment of cell viability, cell functionality using a GSIS test, MTT test and histological evaluation. All tests were performed on β -cells (INS-1E).

RESULTS: The evaluation of bioinks precursors began with their bioprinting capabilities. All 8 variants was possible to bioprint stable constructs. Correspondingly, the pressure was 6-48kPa, and the temperature was 16-25°C. The printing speed was changed and ranged from 5-20mm/s. The next step was to evaluate the cytotoxicity of the analyzed bioinks. The least favourable results were obtained for variants above 13% dECM content. The most positive results for the viability were shown for bioinks with a dECM content of up to 11%. The obtained results showed a significantly higher percentage of viability depending on the tested variant. On the other hand, the addition of dECM in the range of 8-11% showed even 25% higher cell growth than in the group of untreated cells.

Confirmation of the results for the MTT test was obtained during the GSIS analysis. The functionality of beta cells was highest in the case of the bioink containing up to 11%. However, the best results were obtained for the variant of 5% and 8% dECM content. Histological analysis confirmed the advantage of bioinks up to 11%, showing a 3-fold increase in the insulin signal in these bioinks. In these analyzes, the 5% and 8% variants also showed a stronger signal for insulin secretion.

DISCUSSION & CONCLUSIONS: The experiments carried out in this study prove that the appropriate concentration of dECM, which is the natural environment for a cell, must be properly validated in the case of tissue engineering and selected for a given type of cell. In the case of beta cells, it should not exceed a total of 10% of the dECM. It should be remembered that the ECM serves as a physical protection for cells in the native environment, and helps in various biological functions.

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Keywords: Biomaterials, Decellularised matrices

PP-288

In vitro* biocompatible 3D marine-based scaffolds using jellyfish collagen or squid chitosan as matrices and squid chitin nanocrystals as *nanofillers

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INTRODUCTION: Recently, we have demonstrated that chitin nanoforms are promising natural *nanofillers* for biomedical applications.^{1,2} In particular, beta nanochitin is a promising nanoform to make biomaterials with enhanced properties, although this isoform is less studied due to its softer configuration, compared to alpha isoform. The aim of this study was to synthesize 3D scaffolds using beta chitin nanocrystals (β CHNC) as *nanofillers* in marine collagen or chitosan matrices and, to evaluate their effect on the ensuing scaffold's properties and biocompatibility for its application in Tissue Engineering.

METHODS: β CHNC were *in house* isolated following previously reported method,³ and their morphology was characterized by AFM microscopy. The 3D scaffolds of commercial jellyfish collagen or squid chitosan were synthesized by freeze drying method, incorporating or not β CHNC, and crosslinked with EDC or genipin, respectively. Chemical composition was assessed by ATR-FTIR spectroscopy, swelling tests were carried out and the morphology was analyzed by SEM microscopy. *In vitro* cytotoxicity was determined using L929 cells following ISO 10993 standard, and cell adhesion was evaluated by SEM microscopy.

RESULTS: The isolated β CHNC showed characteristic beta chitin bands in ATR-FTIR spectra, and sphere-like morphology ($D: 38.5 \pm 4.9$ nm). The presence of β CHNC into the 3D scaffolds was confirmed by ATR-FTIR analysis and SEM images. The highly macroporous scaffolds underwent a decrease in the pore size after β CHNC addition (1.12 and 1.71-fold decrease for collagen and chitosan, respectively). Interestingly, the swelling capacity increased in collagen scaffolds (6.45-fold) but decreased in chitosan scaffolds (1.75-fold). Regarding biocompatibility, L929 cells cultured in extractive medium from scaffolds prepared with *nanofillers* showed high viability and good attachment in cell adhesion studies.

DISCUSSION & CONCLUSIONS: Sphere-like shaped β CHNC were homogeneously incorporated into both marine matrices. This caused a significant decrease in the pore size and swelling capacity of the chitosan scaffolds, while the collagen-based ones underwent a significant increase in swelling capacity, which may be related to the strengthening of the structure. Both matrices showed high cytocompatibility, and cell adhesion was favored after the incorporation of β CHNC. Therefore, the novel biomaterials developed with β CHNC as *nanofillers* show suitable properties for their use in Tissue Engineering applications.

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Keywords: Polymers - natural / synthetic / responsive,

PP-289

***In-vitro* Studies of 2D Orthopaedic Implants with Cell Instructive Nanotopographies**

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INTRODUCTION: Demand for total hip (THR) and knee replacements (TKR) is increasing worldwide, due to degeneration of subchondral bone, a result of ageing populations and active lifestyles. While the success rate of these implants is relatively high, many fail within 10 years. The two leading causes are aseptic loosening (due to poor osseointegration) or infection (due to bacterial biofilm formation). Although certain topographies are bactericidal, they do not support osteogenesis on their own and require additional coatings. Here, we use plasma poly(ethyl acrylate) (pPEA) polymer coating on surfaces to spontaneously organize fibronectin (FN) and deliver bone morphogenetic protein-2 (BMP2) to improve human mesenchymal stem cells (hMSCs) adhesion and osteogenesis. Simultaneously, the designed patterning and pPEA+FN+BMP2 active coatings have been developed to reduce biofilm formation.¹

METHODS: Nano-patterned titanium surfaces were prepared by etching in sodium hydroxide for 2h or 16h producing nanosurface 1 (NS1) and nanosurface (NS2), yielding structures of 400 and 500 nm in height, respectively.

pPEA was used to coat titanium surfaces, followed by FN and BMP2 treatments. The viability and adhesion quality of primary hMSCs was tested using immunofluorescence staining and colourimetric assays. Antimicrobial properties of coated and uncoated surfaces were challenged using Gram-stained negative bacteria *Pseudomonas aeruginosa*. The viability of hMSCs co-cultured with *pseudomonas aeruginosa* on titanium surfaces was evaluated, as well as the hMSCs attachment to the titanium surfaces and their osteogenic differentiation potential.

RESULTS: Nano-patterned titanium surfaces treated with active coatings such as PEA+FN+BMP2 offer great potential to grow viable hMSCs and sustain osteogenic differentiation. Furthermore, both topographies showed indication of antibacterial potential, corresponding with previous studies prepared using high aspect ratio nanospikes.² Moreover, the exposure of hMSCs to bacteria caused no significant change in the hMSC viability or adhesion features in short-term co-culture. Ultimately, conditions were more favourable for the titanium surfaces with nanopatterns.

DISCUSSION & CONCLUSIONS: This study showed the potential for the implementation of nanotopographical surfaces to address poor osseointegration on bone implants and bacterial biofilm formation after THR and TKR. Coated topographies offer better support for hMSC adhesion and differentiation versus uncoated surfaces. This finding is of importance given that primary cells need to be recruited for successful osseointegration. Moreover, biofilm formation was decreased when bacteria was seeded on NS1 and NS2 patterned surfaces, aiding hMSC colonisation onto the titanium surface.

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Keywords: Biomaterials, Differentiation

PP-290

Biomaterials for cell desiccation and storage: a feasibility and biocompatibility assessment for in vivo translation

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INTRODUCTION: Appropriate storage, transport and delivery of cells is essential for the development and clinical use of veterinary and human cell-based therapeutics. However, current standard preservation procedures (i.e. liquid nitrogen) are logistically and financially challenging for large scale storage or distribution in addition to limitations such as use of cytotoxic compounds (i.e.DMSO), and ice nucleation/crystal formation.

The alternative preservation hereby proposed is the storage and transport of cells in low water environment, supra-zero temperature and within biomaterial as protective scaffolds. This approach would make cell-based research and therapies more accessible to hindered locations such as the research facilities in the International Space Station, or in developing regions, and zones of conflict. The current study includes a compatibility assessment of desiccated-rehydrated animal (i.e. gelatin) and plant-based candidate biomaterials (i.e. alginate, and nanofibrillar cellulose) for its potential use as therapeutics delivery vehicles in vivo.

METHODS: Following the initial characterisation of the thermo-physical and morphological profiles, and monoculture biocompatibility; in this study the candidate biomaterials have also been assessed for their cell growth-support and protective storage of multicellular structures under low water environments, diffusion profiles using an in vitro permeation test, and impact following rehydration on the growth and development of a vasculature network using the chicken chorioallantoic membrane (CAM) assay.

RESULTS: The results of this study showed that survival of desiccated multicellular structures imbedded in candidate biomaterials was comparable in plant-based materials compared to animal derived candidates. In addition, survival rates and metabolic activity in the longer-term post rehydration were dependent on parameters such as environmental temperature while drying, water removal method and duration, and matrix formulation (i.e. biomaterial dilution and exogenous protectant supplementation). Differences in diffusion profiles became apparent according to biomaterial type and highlighted the importance of matrix formulations and supplementation during the process of desiccation. Finally, analyses of the vasculature network in the presence of the candidate materials showed comparable results to the control, hence desiccation and rehydration of materials does not affect angiogenesis and therefore future potential use in vivo.

DISCUSSION & CONCLUSIONS: Overall, the systematic experimentation performed in this study highlighted the versatility of biomaterials as protective scaffolds for multicellular structures in the context of low water environments. What is more, optimal vessel formation as seen from CAM assay results point towards good integration abilities of rehydrated materials, and thus feasibility of safely using this technology in the future for the delivery of cell-based technologies/therapeutics to locations where liquid nitrogen-based transport is impracticable.

Keywords: In vitro microenvironments, Hydrogels and injectable systems

PP-291

Multifunctional antibacterial keratin-polycaprolactone based electrospun nanofiber dressing for skin tissue engineering application

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INTRODUCTION: To replicate and restore the functions of extracellular matrix of native tissue electrospun nanofibers gained huge attention among the scientific world. Keratin, rich in cysteine residues, showed potential usage in biomedical applications [1]. The present study aims to design keratin-based electrospun dressing to reduce the bacterial burden in wound healing application. The beadless nanofiber scaffold was fabricated using optimal Keratin(K)/Polycaprolactone (PCL) solution.

METHODS: In this study, keratin was isolated from chicken feather by reducing method and confirmed with SDS-PAGE and FTIR. K/PCL composition spinnability factor was assessed by rheological as well as conductivity measurements. A spun K /PCL nanofiber chemical composition was confirmed by FTIR spectra and XRD. Further, mechanical and surface properties were studied. To validate the cell-scaffold interactions, cell proliferation was evaluated through MTT assay.

Antimicrobial potential of nanofibers were assessed in different bacterial strains.

RESULTS: Nanofibrous architecture and morphology was investigated by FESEM where the smoother nanofiber were observed with diameter in the range of 170-350 nm. FTIR and XRD confirmed the presence of keratin protein and its interaction with the PCL. The porosity of electrospun nanofiber found to be in the range of 30% to 70% via liquid displacement method. Furthermore, reinforcement of keratin has improved the antibacterial property, important for wound dressing applications. The fabricated keratin based nanofibers showed antimicrobial properties against S.aureus. No hemolytic effect were observed in human red blood cells (hRBCs) in contact with nanofiber. In vitro experiments confirmed the cytocompatibility, excellent bioactivity which promotes cellular adhesion and proliferation of fibroblast.

DISCUSSION & CONCLUSIONS: Keratin is rich in cysteine residue due to which antibacterial property is attributed to the nanofiber. Cellular compatibility of the nanofiber is due to the presence of RGD and LDV residue. Hence, confirmed the feather keratin-based electrospun nanofiber is a promising choice for futuristic use in wound healing for restoring the damaged tissue.

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Keywords: Biomaterials, Skin

PP-292

Multifunctional Gallium Doped Bioactive Glasses: a targeted delivery for antineoplastic agents and tissue repair against osteosarcoma

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INTRODUCTION: Osteosarcoma (OS) is an aggressive form of cancer in hard tissue. Treatment options are limited as the diagnosis and prognosis are complicated (1). Gallium (Ga) doped bioactive glasses (BG) are a promising platform for treatment of bone cancer as Ga ions can suppress cancer cells due to their antineoplastic properties while the controlled release of calcium (Ca) and phosphorous (P) from BGs can stimulate new bone regeneration (2). Here we present the physical and biological characterisation for a series of novel Ga based BGs evaluating their efficacy for bone cancer treatment.

METHODS: We have fabricated Ga doped BGs with increasing Ga content (0-5 mol %). The cytotoxic effects on Saos-2 (osteosarcoma) cells were evaluated using MTT and LIVE/ DEAD assays. Live cell imaging was also performed using IncuCyte imaging system to investigate OS cells migration through scratch wound assays. Normal human osteoblast (NHOst) cells were used as a control to ensure the materials developed were not dangerously cytotoxic to normal health cells. Dissolution of active ions (Ga, Ca, P) were recorded using Induced Couple Plasma Mass Spectrometry for each of the glass compositions. Apatite formation was investigated using X-ray diffraction, FTIR and EDS.

RESULTS: MTT assay results for OS (Saos-2) and NHOst cells cultured using conditioned media from BG containing 0-5 mol % Ga doped powder and a complete kill of osteosarcoma cells was achieved after 10 days. In contrast, over half the NHOst survived the process. The results obtained from LIVE/DEAD assay was in agreement with MTT results. Scratch wound assay results demonstrated a significant inhibition of OS cell migration following exposure to Ga doped BG. The release rate of ions helped quantify the levels of gallium ions needed to kill the osteosarcoma cells whilst still remaining within the therapeutic region whereby the cytotoxic effect against NHOst was acceptable. Apatite formation was observed using X-ray, FTIR and EDS.

DISCUSSION & CONCLUSIONS: The results demonstrated an important step forward in the creation of potential bone graft for OS treatment post-surgery. The method of delivery for this platform is multi-directional as the controlled and targeted release of Ga ions with anti-tumour properties from BG alongside with the release of phosphate and calcium ions with the purpose of bone regeneration. The cytotoxic effect of Ga doped BG on the OS cells provide valuable data for management of bone cancer.

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Keywords: Cancer, Bone and bone disorders (osteoporosis etc)

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Sonic micro-fragmentation exposes extracellular matrix components to increase the osteogenic potential of human decellularized bone tissue

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INTRODUCTION: Reconstruction of bone defects is still challenging in orthopedic surgery. Bone decellularized extracellular matrix (dbECM) has been developed as an allogeneic alternative to autografting due to its composition (collagenous and non-collagenous protein proper of native tissues) [1]. However, the application dECM is restricted by scarce availability, limited manageability, and its biological performances that are substantially lower than autograft bone. In this study, we developed a process to treat human bone to produce smaller particles with improved osteoinductivity based on an ultrasonication procedure.

METHODS: Bone chips were produced from human bone harvested from cadavers in Rizzoli Orthopedic Institute tissue bank. The bone was fragmented with a ball miller, sieved, treated to remove cells and finally processed with a probe ultrasonicator. In this last step, the bone particles explode, revealing their internal organic phase. This was confirmed by SEM and confocal imaging. A response surface method was used to develop empirical model relating the sonication power, the process time, and the duty cycle to the properties of the bone particles produced. The particle features (dimension and morphology) were extracted by a deep-learning image segmentation. The particles were embedded in a silk hydrogel matrix (to allows their eventual use in clinic) and then tested in-vitro for 14 days, in term of cell proliferation and ALP activity.

RESULTS: We demonstrated by computing a correlation matrix the inverse correlation of the osteoinductivity with the particle's mean area (and equivalent volume) and its direct correlation with the total exposed surface. The treatment was proven to be effective in reducing the particles dimensions and to expose the organic components. The organic component was probably the reason behind the improved osteoinductivity. The ultrasonication was modelled by a rigorous RMS revealed a general decrease of the particles dimensions with the increment of the sonication power and processing time while the effect of the duty cycle resulted to be not significant. The extrapolated empirical equations allowed us to control the particles mean dimensions.

DISCUSSION & CONCLUSIONS: The process here proposed is fast, economic, and scalable to large production. In addition, the incremented osteogenic activity and the decrease in the particles size may increase the number of treatable patients with the same raw bone tissue.

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Keywords: Decellularised matrices, Composite materials

PP-294

Progressively Self-Stiffening Hydrogels for Tissue Engineering

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INTRODUCTION: Living systems are dynamic in nature. In many biological scenarios such as tissue healing or cancer progression, the stiffness of the extracellular environment changes over time. For example, during bone healing, the hematoma gradually transitions from a soft (~0.5 kPa) to hard (~100 MPa) callus, a stiffening process that is believed to influence the behaviour of multiple cells in the healing process¹. Beyond regenerative scenarios, cancer progression also presents a progressively stiffening milieu of up to 20-fold as the cancer environment remodels and evolves.² Therefore, there is potential utility in materials that can recreate these progressively stiffening environments to conduct more physiologically relevant mechanobiological studies of the processes that occur in development tissue.

METHODS: We have developed a co-assembled hydrogel based on hyaluronic acid tyramine (HAT) and Laponite® (Lap) (Figure 1A) with the capacity to progressively stiffen for a determine period of time. The hydrogel is formed through electrostatic and H-bonding interactions between positively charged Lap rim and negatively charged HAT. Lap face with negative charge can be bridged by cations present in DMEM, which slowly diffuse into the hydrogel, gradually increasing the hydrogel stiffness. Hydrogels were characterized by rheology and different spectroscopic techniques including FTIR, zeta potential, NMR, and XPS.

RESULTS: The resulting hydrogel exhibited tuneable progressive stiffening properties from 0.8 kPa to 7.5 kPa within a ~48 h period (Figure 1B). Human bone marrow mesenchymal stromal cells (HBMSCs) were seeded and cultured on static (i.e., pre-stiffened gels, displaying a consistent stiffness of 7.5 kPa) and dynamic (naïve gels, displaying an increasing stiffness of 0.8 – 7.5 kPa over 48 h). After 3 weeks of culture, HBMSCs exhibited significantly enhanced osteoblastic phenotype when growing on the dynamic hydrogels compared to the static ones (Figure 1C).

DISCUSSION & CONCLUSIONS: The results demonstrate that even a short (~ 2 day) hydrogel stiffening period has a marked effect on osteoblastic phenotype. These hydrogels represent an exciting physiological model to investigate cellular mechanosensitive responses to progressive changes in the microenvironment with applications in hard and soft tissue regeneration.

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Keywords: Biomaterials, Biomechanics / biophysical stimuli and mechanotransduction

PP-295

in vitro biological activity of stront-doped bioglass for multifunctional chitosan composites

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INTRODUCTION: The field of biomaterials is constantly evolving and searching for innovative solutions [1][2]. However, the functional properties of the emerging medical devices are still far from expectations, especially in the field of osseointegration. Ions released from the biomaterial (eg. Sr²⁺) generate a favourable microenvironment that facilitates cell proliferation. Thanks to which bone cells are stimulated to create a connection with the implant. The aim of our the study was to obtain SrO-doped bioactive and bactericidal glasses for multifunctional composites using the sol-gel method.

METHODS: The developed chemical formulations of bioglass belonged to the ternary CaO-SiO₂-P₂O₅ system. Bioglass of 70 wt% SiO₂, 5 wt% P₂O₅ and 25 wt% CaO was used as a basic material. As for the composition of SrO-doped bioglass, 2 wt% or 5 wt% of CaO in basic glass was replaced with SrO. Having performed the reaction mixtures from sol to gel and after the drying process was completed heat treatment at 650°C for 15 h The samples were prepared in two grain sizes.

The biological tests were carried out using Human osteoblast cell line hFOB 1.19 in accordance with ISO 10993. Cell proliferation and cytotoxicity was determined by LDH and WST-1 tests. To determine antimicrobial properties of bioglasses cell cultures *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used.

RESULTS: No result of the lactate dehydrogenase (LDH) activity test on osteoblasts incubated with the test glasses exceeded the threshold of 30%. The results of the WST-1 assay used to evaluate the proliferation of hFOB1.19 cells are shown that all tested bioglasses exceeded the threshold of 70%.

DISCUSSION & CONCLUSIONS: This results allows the tested bioglasses to be classified as a material that does not show cytotoxic activity, regardless of the particle size. Whereas the observed reduction results of microorganisms indicate a higher sensitivity of *Pseudomonas aeruginosa* bacteria compared to *Staphylococcus aureus* to bactericidal agents present in contact with the tested bioglasses.

The tested bioglasses were classified as a non-cytotoxic material. Stront-doped bioglass showed increased proliferation compared to a basic material.

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Keywords: In vitro microenvironments, Composite materials

PP-296

Silk Based Photocrosslinkable Growth Factor Laden Injectable Hydrogel for Meniscus Defects

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INTRODUCTION: A facile treatment approach that could be adopted to treat irregularly shaped tears of the avascular knee menisci by virtue of a mechanically resilient injectable hydrogel, loaded with autologous growth factors, could stabilize the damaged meniscus tissue. Hydrogels are required to possess apt mechanical compliance as well as enhanced bioactivity and sustained release of growth factors to promote the healing of the meniscal tissue in the avascular and vascular regions. Here, we have developed a silk-based platelet rich plasma lysate (PRP) loaded injectable hydrogel to treat major and minor meniscal tears.

METHODS: Silk methacrylate (BMMA) and gelatin methacrylate (gelMA) were synthesized and blended with polyethylene glycol dimethacrylate (PEGDMA), PRP and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) to form hydrogels for encapsulation of human wharton's jelly derived mesenchymal stromal cells (hWJMSCs). The acellular hydrogel was characterized for its rheological, swelling, degradability, porosity and mechanical properties. Further, the biological and functional assessment was performed on cell laden hydrogels, along with in vitro and in vivo biocompatibility of the acellular hydrogels.

RESULTS: Injectable hydrogel composition showed optimal crosslinking within 60 seconds in presence of visible light. The hydrogel composition showed suitable shear thinning and gel strength before crosslinking and apt swelling, long-term degradability, compressive and tensile mechanical durability post-crosslinking. It also demonstrated sustained release of platelet rich plasma lysate over long duration. Biological characterization, post encapsulation within the hydrogels, using hWJMSCs showed apt proliferation and cellular migration. Gene expression profile of aggrecan, sox-9, collagen I and collagen II showed fibro-chondrocytic differentiation. Concomitant deposition of sulphated glycosaminoglycans (sGAG) was seen through biochemical estimation. Collagen I, collagen II and aggrecan deposition was found in co-stained histological sections showing their uniform deposition. The hydrogels also demonstrated excellent immuno-compatibility in vitro when presented to murine as well as human derived macrophages. Also, in vivo immuno-compatibility was found to be apt when implanted in rabbit subcutaneous pockets.

DISCUSSION & CONCLUSIONS: The developed injectable hydrogel composition could be used to potentially treat different kinds of radial, full thickness and longitudinal meniscal tears in vivo owing to its rapid photo-crosslinking nature, mechanical resilience, growth factor reservoir and regenerative potential.

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Keywords: Biologics and growth factors, Knee

PP-297

Silk based 3D printed human skin model for high-throughput screening of topical formulations

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INTRODUCTION: A well-defined skin model platform is an utmost need for efficacy assessments of newly developed topical and transdermal formulations. Recent studies mostly rely on the use of animal models for this purpose. The use of animals for pre-clinical testing is rightly criticized and strongly questioned for scientific and ethical reasons. As an alternative, several strategies have been explored for the fabrication of biomimetic skin models, but currently available collagen-based skin models fabricated through multistep processes are costly, creating a gap between need and availability. Here, we present a 3D bioprinted human skin model with a functional transwell system which can be utilized to evaluate skin sensitizing effects of topical formulations.

METHODS: Bioactive photocrosslinkable bioink was formulated by blending silk fibroin methacrylate (SilkMA) gelatin methacrylate (GelMA) and photoactivated platelet releasate (PPR) with photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) at optimum concentrations. Physicochemical and rheological characterizations of the formulated bioink were performed. Next, the bioink was utilized to print pre-vascularized skin construct with biomimetic epidermal layer supported by an artificial basement membrane of in-house built transwell system. The printed construct was matured for 14 days in air-liquid interface (ALI) culture and characterized through detailed immunostaining. Further, the model was evaluated through permeability and skin irritation tests to assess its aptness in screening applications.

RESULTS: The developed silk-based bioink showed excellent cytocompatibility and suitable physicochemical and rheological attributes for extrusion-based printing. After computer-aided bioprinting of the skin construct, immunofluorescence staining of the matured model showed a well-defined keratinocyte layer and a stabilized fibroblast-stretched dermis innervated with human vascular endothelial cells. The innovative micro-architectural design and use of alternative composite biomaterial have overcome the shortcomings related to poor mechanical stability and contraction of the collagen-based skin model used as control group in the study. The improved barrier-function property of the printed model was quantified through the permeability assay using fluorescently-labelled molecules. The fabricated model was also able to distinguish between irritative and non-irritative chemicals, demonstrating its suitability as an in vitro screening platform for pre-clinical tests.

DISCUSSION & CONCLUSIONS: Bioprinting technology aided in rapid single-step fabrication of the 3D-engineered skin model. Further, the results of the efficacy assessments of the model support its potential to be utilized as an automated platform for high-throughput screening of pharmaceuticals and cosmeceutical agents.

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Keywords: 3D printing and bioprinting, In vitro microenvironments

PP-298

Optimization of the hydrogel injectability for the pain-minimized administration

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INTRODUCTION: Application of advanced hydrogels for tissue reconstruction as a medical device or a therapeutic agent requires a careful administration with a controlled injection rate to avoid excess pain and reach the intended outcomes. For non-Newtonian fluids at manual injection it is challenging as in general there is limited knowledge of the rate at specific needle, depth and angle, as well as opposing underlying tissues. A "safe" level of internal pressures for soft tissues is considered to be below 5~10 kPa (preferably <4 kPa), and for a short-term 13~20 kPa: once it exceeds the vascular pressure differential, blood flow will be reduced and may temporarily cease, potentially leading to local anoxia and localized ischemia [1].

In this work a new method has been developed to uniquely characterize hydrogels injectability without a need of the particular rheological model or assumptions, allowing predictive estimation of the programmed injection requiring no explicit "smart needle" rate control.

METHODS: In the demonstrated example, the method is shown for own experimental data of the amount of hydrogel (μL) injected via a defined nozzle (needle gauge), per applied local pressure (kPa), which is complemented of testing the same hydrogel and needle setup into the skin phantom. Time-invariant values such as viscous stiffness of the gel in injection conditions and its memory value have been extracted with a patented method [2].

RESULTS: Experimental data clearly revealed a non-linearity of the flow kinetics with time and applied pressure. The curves of viscous stiffness and memory are almost linear for all applied pressures (numbers in kPa). It also shows that numeric values are changing with applied pressure in a non monotonic way, disclosing that there are might be different limiting phenomena affecting the flow. The hydrogel injectability map has been constructed showing the gel in the syringe setup faces friction, flow resistance and possibly no-slip effects at low pressures when memory values are much less than unity.

DISCUSSION & CONCLUSIONS: The method developed can assess the injectability properties vs. specific injection system conditions which usually are very different from standard rheology setups, giving inconsistent outcomes due to uneven contact, inertia and elastic instability effects, fitting to improper assumed models, lack of proper loading history assessment, etc. New method allows bypassing these limitations and can be directly deployed in optimized injection systems design compliant to MDR 2017/745.

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Keywords: Innovation, Translation and commercialisation (inc. clinical trials and regulatory approval)

PP-299

Antimicrobial hyaluronan based hydrogels for anastomotic leak prevention

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INTRODUCTION: Hyaluronan based *in situ* gellable hydrogels were suggested as suitable materials for the prevention of post-operative adhesions. The aim of this study was to develop an injectable hydrogel with antibacterial properties for prevention of dehiscence of colorectal anastomosis. The hydrogel should cover anastomosis to prevent organ adhesions and anastomotic leak. The presence of antimicrobial agent should decrease the risk of post-operative infection.

METHODS: Hyaluronan-tyramine conjugate (HA-Tyr) was synthesized following previously reported protocol [1]. The structure and molecular weight of the HA-Tyr conjugate were determined by ¹H NMR and SEC-MALLS, respectively. Hydrogels were prepared by a horseradish peroxidase (HRP) mediated crosslinking reaction [1]. Triclosan/hydroxypropyl- β -cyclodextrin inclusion complex (TCS/CD) was used as the antimicrobial component. Gelation time and viscoelastic properties of hydrogel were evaluated by TA Instruments AR-G2 rheometer. The applicability of the material, amount of material needed for the procedure, rate of the hydrogel gelation and degradation *in vivo* were evaluated during preliminary pig study involving three animals.

RESULTS: TCS is a hydrophobic drug poorly soluble in water. It is well known in the field of coating implantable devices. The TCS/CD inclusion complex was prepared to solubilize the drug in water to provide a homogenous distribution of TCS in the hydrogel. Previously we found that hydrogels containing at least 0,8 mg/ml of TCS exhibited sufficient antimicrobial efficacy towards tested microorganisms.[2]. Hydrogels containing 1 mg/ml TCS were used for preliminary *in vivo* study. During *in vivo* pig study a laparotomy was performed in the experimental animals. Solidification of hydrogel took approximately 5 min. From 20 to 30 ml of hydrogel were used to isolate rectosigmoid colon from the rest of the peritoneal cavity.

DISCUSSION & CONCLUSIONS: A surgical second-look revision was performed 14 days after the primary surgery. In the second-look surgical revision, the gel was found to be in place. The experimental animals had no signs of postoperative complications or bowel obstruction. Furthermore, we found no adhesions in this group. On histological examination, we found no inflammatory processes or pathological changes in the serous layer of the bowel. Designed hydrogel is promising material for preventing of anastomotic leak following colorectal surgery.

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Keywords: Hydrogels and injectable systems, Infection

PP-300

Microencapsulation of mesenchymal stem cells in covalent hydrogel for cell therapy

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INTRODUCTION: Recently, mesenchymal stem cells (MSCs) have been proposed as a relevant therapeutic approach for cell therapy. They can secrete anti-inflammatory, immunomodulatory, and regenerative factors and have been injected into diseased joints. Considering low cell viability after injection and cell leakage outside the injection site, the encapsulation of MSCs in hydrogels will protect the cells and provide a suitable 3D microenvironment supporting their biological activities. For cell encapsulation, sodium alginate is the most studied and characterized polymer. Its cross-linking with calcium ions has been widely investigated, but the presence of sodium in the joint might alter its mechanical properties. To remedy this, the covalent cross-linking of the alginate with the SPAAC (Strain Promoted Alkyne-Azide Cycloaddition) system allows control of the mechanical properties of the hydrogel and better stability in time. This work aims to develop a hydrogel using a micromolding technique to generate small particles to be injected into a joint.

METHODS: The SPAAC hydrogels were obtained by functionalizing alginate with a molecule presenting an azide (PEG-N3) or an alkyne on a strained ring (bicyclononyne, BCN). After a single-step reaction synthesis, the alginate components (alginate-BCN 0.2Eq and alginate-N3 0.5Eq) were mixed to obtain hydrogels under physiological conditions. Rheological tests of gelation time, stability studies in Dulbecco's Modified Eagle Medium, and stiffness tests were performed to characterize hydrogel formation. We then succeeded in micromolding the covalent alginate using polydimethylsiloxane molds (diameter 150 µm, height 100 µm).

RESULTS: A shorter gelation time was evidenced with the SPAAC-alginate hydrogel at 37°C versus room temperature. A minimum of 30 minutes of cross-linking at 37°C was necessary to prevent the hydrogel from dissolving. Then, we demonstrated that bulk and micromolded hydrogels were stable over time under physiological conditions after one-hour cross-linking at 37°C. Surprisingly, SPAAC-alginate hydrogel stiffness increased over time until reaching its final mechanical properties after 24 hours. Furthermore, we evaluated the diffusion of pro-inflammatory cytokines (TNF-α and IFN-γ) through this hydrogel. While TNF-α diffused well through the SPAAC-alginate hydrogel (70% in 3 days), IFN-γ did not diffuse well through it (5% in 3 days).

DISCUSSION & CONCLUSIONS: A murine cell line (L929) was then encapsulated in a SPAAC-alginate bulk hydrogel. Live/dead confocal imaging confirmed the cell viability after encapsulation. Further in vitro experiments are under investigation to characterize the cytocompatibility of human MSCs

encapsulated in this covalent hydrogel.

Keywords: Cell therapy, Biomaterials

PP-301

Immediately implantable graphene oxide-laden glycol chitosan / hyaluronic acid based hydrogels for in situ bone therapy

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INTRODUCTION: Graphene oxide (GO) incorporated hydrogels have received great attention and have shown excellent potential for use in the field of bone tissue engineering due to their unique osteogenic functionalities. However, current hydrogel systems are limited in their ability to provide an appropriate amount of GO to the lesion area.

METHODS: To overcome this issue, we designed a GO incorporated glycol chitosan (gC)-hyaluronic acid (HA) injectable hydrogel system via a simple oxidation technique. Through oxidation, aldehyde groups were introduced onto the HA. Blending this with gC allowed for the formation of an aqueous hydrogel matrix.

RESULTS: Physico-chemical characterization demonstrated that the gC/oHA/GO hydrogel matrix exhibited robust mechanical properties and stability. The in vitro and in vivo results showed that GO incorporated injectable hydrogels enhanced bone tissue regeneration as compared to control injectable hydrogels.

DISCUSSION & CONCLUSIONS: Therefore, our results indicate that our injectable hydrogel system could be used for delivering GO. This material may serve as an excellent tissue scaffold for use in treating bone defects.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-302

Silk fibroin/hyaluronic acid-based hydrogels: a 3D printable hydrogel for vocal fold tissue engineering

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INTRODUCTION: Vocal fold (VF) defects caused by various environmental factors and pathological conditions remain among the foremost therapeutic challenges that detrimentally affect the daily lives of suffering individuals. It is estimated that up to 9% of the general population suffer from a voice disorder at one point over their lifetime. The most severe disorders affecting voice are those in which large defects, such as cysts, nodules, or cancerous tumors, need to be removed. When large lesions are surgically resected using cold knives or lasers, larger voids are frequently created. VF has been shown to be unable to restore or regenerate itself. Hence, tissue engineering approach will be needed to promote the formation of a healthy and functional vocal fold. Among various therapeutic techniques, in-situ printing of a hydrogel-based construct seems to be a viable approach for repairing relatively large vocal fold defects. To mimic the nature of a native extracellular matrix, the in-situ printable hydrogel proposed in the current project will be based on a polysaccharide, hyaluronic acid (HA), and a protein, silk fibroin (SF).

METHODS: In order for the hydrogel to meet the delivery technique requirements, including fast gelation and strong tissue adhesion, HA chains were grafted with dopamine groups. To do so, HA chains were first oxidized to present aldehyde groups on their backbone and then dopamine groups were grafted onto aldehyde groups via Schiff base reaction. The HA/SF hydrogels were instantly formed through Fe^{3+} - dopamine coordination and such physically crosslinked hydrogels were further strengthened through a chemical crosslinking between silk fibroin's amine groups via genipin.

RESULTS: NMR results conformed the proper modification of HA. Prepared hydrogels were then characterized in terms of their physical, mechanical, rheological, and biological properties. SEM images showed that prepared double-network hydrogels have microporous structures which is due to the instantaneous tris-coordination between dopamine groups and iron ions. Such microporous structures are advantageous for promoting cell infiltration through the scaffold. Rheological measurements revealed that the hydrogels have self-healing property which is because of the reversible physical crosslinking. Other results also approved that the proposed hydrogel is highly suitable for the target application.

DISCUSSION & CONCLUSIONS: Our ultimate goal is the permanent repair of injured vocal fold tissue using printed biomaterials for lesion specific applications. The results revealed that our proposed hydrogel with fast-forming, self-healing, pore-forming and strong adhesion properties, can fully meet the application requirements and will be a promising candidate for in-situ vocal fold repair.

Keywords: 3D printing and bioprinting, Hydrogels and injectable systems

PP-303

3D Microenvironments of gelatin and elastin like recombinamers for the culture of hepatic cells

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INTRODUCTION: Drug-induced liver injury (DILI) is a health and economic problem in terms of patient morbidity and mortality and drug withdrawal from the market by pharmaceutical industries¹. New 3D *in vitro* culture models for the precise prediction of DILI events in preclinical trials would be a prominent solution. We propose an advanced family of injectable hydrogels that combine gelatin (Gel) and elastin like recombinamers (ELR) as interpenetrated network and functionalized with hepatic growth factors (HGF) as a hepatocyte culture platform for the future prediction of hepatotoxicity events to prevent consequences of DILI.

METHODS: IPN hydrogels with different volumetric proportions of Gel and ELR were synthesized (Gel/ELR):100/0, 75/25, 50/50, 25/75, 0/100. Two different crosslinking mechanisms took place for hydrogel formation: enzymatic reaction for Gel and click chemistry for ELR. The mechanical properties were measured by rheology. Morphology was characterized by scanning electron microscopy and thermal analysis. Equilibrium water content (EWC) and hydrogels stability were evaluated. Heparin attachment and (HGF) binding were analysed by fluorescence. HepG2 cells were cultured within the hydrogels.

RESULTS: Pure Gel (100/0) had a shear storage modulus (G') of 201 ± 51 Pa and pure ELR (0/100) of 1506 ± 241 Pa after gelling and 24 h swelling in culture media. Intermediate values were obtained for the Gel/ELR. All hydrogels showed the typical honeycomb like morphology and the different pore sizes correlated with the crosslinking densities and swelling. IPN formation gives rise to phase-separated systems characterized by the presence of two glass transition temperatures. The mixture 50/50 was able to retain 95 ± 2 % of heparin. Hydrogels containing heparin showed capacity to bind HGF, 77 ± 3 % was retained for the mixture 50/50. Encapsulated HepG2 cells were viable and showed an improved hepatic functionality compared to monolayer cultures.

DISCUSSION & CONCLUSIONS: The higher the elastin content, the higher the mechanical properties of the mixtures. The appropriate combination of Gel and ELR provides mechanical properties similar to those of liver. The incorporation of HGF able to be presented to cells from the hydrogel matrix is demonstrated.

Gel/ELR hydrogels seem suitable 3D systems for hepatocytes culture. Future experiments will explore their potential for hepatotoxicity studies.

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Keywords: Biomaterials, In vitro microenvironments

PP-304

Characterization of Collagen I, II, and III Blended Hydrogel Polymerization Kinetics for Tissue Engineering Applications

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INTRODUCTION: The extracellular matrix (ECM) confers structure to tissues and provides mechanical and biochemical signals to cells. Collagen (col) is the most abundant protein in the body and the main structural component of the ECM. Due to its abundance and a relatively easy extraction, col I is the most common collagen used in tissue engineering applications. However, most tissues are composed of a combination of different collagen types. Blended col hydrogels (containing more than once col type) have been studied, including col I/II hydrogels for cartilage tissue engineering¹ and col I/III hydrogels for cardiac and vocal tissue engineering.² The addition of col II and III for these applications improved biological responses which highlights the importance of chemical cues blended col hydrogels provide. This work aims to characterize contribution of collagen types II and III, and various polymerization temperatures³ on the blended collagen hydrogels for tissue engineering applications. **METHODS:** Blended collagen I/II and I/III were fabricated at ratio of 3:1 collagen I to collagen II or III. Polymerization kinetics were assessed for 25, 30, and 37 °C. Microstructure, mechanical properties, and transport properties were characterized.

RESULTS: Our results demonstrate that blended collagen hydrogels exhibit unique polymerization kinetics, which in turn result in distinct microarchitectures, mechanical properties, and transport profiles. Blended hydrogels polymerize slower compared to pure collagen I gels. Blended col I/III gels polymerized faster compared to collagen I/II blended gels, a result which might be expected as col I and III are often co-polymerized in vivo, while col I and II are rarely found in same tissues.⁴ Blended col hydrogels have altered microstructures, including lower volume occupied by fibrils and larger pore sizes for all temperatures tested. Rheological results demonstrate that mechanical properties for all gel types were the highest for hydrogels polymerized at 25 °C. Transwell macromolecular recovery studies demonstrated that dextrans of various molecular weights recover faster in pure collagen I hydrogels, compared to col I/II and col I/III hydrogels.

DISCUSSION & CONCLUSIONS: Overall, blended col I/II and col I/III hydrogels exhibit properties distinct from pure collagen I gels. These properties can be tuned to specific tissue engineering applications by modulating polymerization temperatures.

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Keywords: Biomaterials, Hydrogels and injectable systems

PP-305

Injectable hydrogel composites based on hydroxyapatite, epsilon-polylysine and hyaluronic acid for bone regeneration

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INTRODUCTION: A functional bone grafting materials should be bioactive, injectable, antibacterial, provide appropriate mechanical properties and act as a drug delivery system. The aim of this study was to develop and investigate bioactive hydrogel composites based on natural biopolymers - hyaluronic acid (HA) and ϵ -polylysine (ϵ -PL), as well as strontium ranelate loaded hydroxyapatite nanoparticles (SrRAN-nHAp).

METHODS: Bioactive hydrogel composites were synthesized by chemical crosslinking using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) crosslinking agents (with ϵ -PL to HA mass ratio of 50:50 wt%, with EDC to NHS molar ratio of 1:1) (based on methodology [1], [2]). SrRAN-nHAp was synthesized by wet chemical precipitation (with 10 wt% Sr) and incorporated into hydrogels systems. The molecular structure, phase composition, microstructure, morphology, rheological properties and drug release kinetics of the fabricated hydrogels composites with SrRAN-nHAp to ϵ -PL-HA mass ratios of 60:40 wt%, 50:50 wt%, 40:60 wt%, and 70:30 wt% were investigated. Drug release profile from SrRAN-nHAp and SrRAN-nHAp/ ϵ -PL-HA composite hydrogels was evaluated in PBS at 37°C for up to 30 days.

RESULTS: Phase composition and molecular structure of the lyophilized hydrogel composites revealed the formation of covalently bonded hydrogels with incorporated SrRAN-loaded HAp nanoparticles. SEM micrographs of the lyophilized composite hydrogels revealed a homogenous and porous structure. μ CT images showed a homogenous distribution of SrRan-nHAp powder particles into the hydrogel matrix. In the first 30 days, the SrRAN-nHAp/ ϵ -PL-HA hydrogel composites with 70:30 wt% showed the highest release of drug up to 36%, and hydrogel composites with 40:60 wt% showed the least release of drug up to 27%.

DISCUSSION & CONCLUSIONS: The developed bioactive hydrogel composites based on SrRAN-nHAp, ϵ -PL, and HA showed promising functionalities for bone regeneration application, such as appropriate viscoelastic properties, sustained drug release profile, high porosity, and swelling degree. Further studies on antibacterial activity and cytotoxicity are required to determine the full biomedical potential of the developed biomaterials.

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PP-306

An adipose derived decellularised extracellular matrix as a biomaterial for tissue engineering of soft tissue

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INTRODUCTION: Vascularised tissue transplants are currently used to reconstruct soft tissue defects caused by trauma, cancer or infection but is not without its limitations and risks. Decellularised extracellular matrix are tissues that have been processed to remove cellular content whilst retaining its network of structural proteins, glycoproteins and adhesion proteins, providing a natural scaffold for cellular attachment, migration and tissue formation, making it a suitable candidate biomaterial for tissue engineering vascularised tissues.

METHODS: Using the method developed by Poon et al (1), we created a decellularised extracellular matrix hydrogel from porcine adipose tissue (pDAT) by applying physical and chemical processing methods to remove lipids and cellular content. DNA from the pDAT and native porcine tissue was extracted using the Qiagen DNeasy kit and quantified using the NanoDrop. Nuclei was visualised with DAPI staining. Liquid chromatography-coupled tandem mass spectrometry was used to characterise the proteome of the decellularised matrix compared to native tissue. Characterisation of tissue generation, angiogenic and immune properties in vivo was assessed using the rodent flow through model(2) and compared against gelatin methacrylate 7.5% w.t. (Gelma), a mixture of Gelma 7.5% w.t. and pDAT, pDAT alone, a commercially available decellularised matrix (Jellagen), as well as a self-assembling synthetic peptide (Manchester Biogel Alpha 2).

RESULTS: We successfully produced a hydrogel with thermal gelling properties. DNA quantification showed a statistically significant decrease in DNA content, with corresponding DAPI staining showing no nuclei staining. Proteomic data shows the presence of various collagens including collagen I and IV as well as extracellular matrix proteins such as fibronectin and laminin remaining in pDAT. Tissue generation was greater in both the decellularised matrixes. The number of blood vessels per area was greatest in the Gelma/pDAT mixture. Jellagen appears to induce the greatest foreign body response.

DISCUSSION & CONCLUSIONS: pDAT shows promise as a biomaterial for tissue regeneration with potential for use in tissue engineering.

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vascular carrier, arteriovenous shunt loop or arteriovenous bundle, has more potential for angiogenesis and tissue generation? *Plast Reconstr Surg.* 2003;112(6):1636-44.

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Characterization and repopulation of decellularized porcine caval vein

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INTRODUCTION: The presence of natural proteins [1] and a decreased risk of infection and thrombosis [2] are the main characteristics of decellularized blood vessel scaffold when compared to synthetic grafts. Tissue decellularization provides a biological matrix preserving native components while removing cellular immunogenic material. Since currently available decellularization protocols are mainly focused on arteries and take more than a day [2], our goal was to develop a fast decellularization procedure for preparation of xenogeneic vein grafts.

METHODS: Infrahepatic caval veins were explanted from anaesthetized pigs and frozen in a cryoprotective solution. After thawing, vessel was ligated to a connector and a tubing system to enable detergents peristaltic circulation. We applied two 1h cycles of 1% Triton X-100 followed by two 1h cycles of 1% sodiumdodecylsulphate to decellularize the vein and then we washed the scaffold first with 15 min cycles of saline. In order to clear the graft from any detergent residues, vessels were kept immersed in a saline buffer for one week with daily change. Human endothelial cells were seeded on the lumen inside a bioreactor.

RESULTS: As shown by hematoxylin and eosin staining of the decellularized caval vein sections, we achieved the fully acellular scaffold in about five hours [3]. The removal of DNA was also confirmed by DAPI staining. We further characterized the scaffold using specific histological techniques to assess the preservation of individual extracellular matrix components (picrosirius red staining visualized in polarized light for collagen, Verhoeff's green trichrome for elastin and Alcian blue for glycosaminoglycans and immunofluorescence for specific collagen types and proteoglycans). Immunofluorescence was also used to visualize the adhesion and shape of the repopulated scaffolds.

DISCUSSION & CONCLUSIONS: In conclusion, our fast protocol resulted in a good quality scaffold. The bioreactor enabled us to recreate the endothelial barrier with cells of human origin and our next step will be the *in vivo* proof of function on a pig model.

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Keywords: Decellularised matrices, Bioreactors

PP-308

Tumor derived extracellular matrix allows the creation of a patient-personalized in vitro tumor

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INTRODUCTION: The extracellular matrix (ECM) represents the major structural component of the tumor microenvironment. The ECM is a non-cellular network of proteins providing to the tumor cells the first environment to proliferate within, to colonize and to migrate from. Indeed, the tumor is a very dynamic microenvironment, the study of the ECM structure is the first step not only to investigate the nature of the tumor scaffold but also to understand cellular, molecular, and immunologic mechanisms of tumor response and resistance. Several well-known biomaterials were proposed to mimic the 3D tumor scaffold. However, the cell adhesion to these biomaterials do not reflect the real tumor cells-microenvironment interactions due to the lack of the complex structure typical of the ECM tumor.

To overcome these limitations, we proposed to recreate a 3D tumor structure using the decellularized matrix of a native tumor as a scaffold to be colonized by a variety of cell types. This could allow the study of the interactions between tumor cells and immune system, and the development of a culture system for the screening of drugs for targeted therapeutic models.

METHODS: Murine B16f10 melanoma cells were expanded and injected subcutaneously to C57Black/6 mice to create a tumor. After two weeks we harvested the tumor and the decellularization process in static and dynamic method was followed by recolonization of the tumor matrix by cells (GFP-MDA and NHI3T3). After 7 days the re-cellularized tumors were recovered and a histological analysis was performed.

RESULTS: The decellularization process removed all the cells from the tissue as shown by the absence of cells and it was not detrimental to the extracellular fibers.

We observed difference according to the different protocols adopted. After 7 days of culture, in static system the cell attachment was limited to the border of the scaffold while in dynamic condition the cells were more integrated into the decellularized matrix. In static decellularization condition by structural histological analysis we observed a collapse of the matrix. This was confirmed by SEM images, that showed fibres twisted on themselves generating a complex net, impenetrable from cells.

DISCUSSION & CONCLUSIONS: The murine melanoma tissue is suitable for the creation of a 3D in vitro tumor microenvironment when decellularization and recellularization are performed in a dynamic system. This method could allow to obtain a patient-personalized tumor, adequate for drug screening that will eventually drive to the more appropriate drug therapy for each patient.

Keywords: Decellularised matrices, In vitro microenvironments

PP-309

Artificial intelligence biosensing approaches for evaluating the effectiveness of corneal decellularization

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INTRODUCTION: Decellularized corneas offer a promising source of replacement grafts. Despite great success in achieving acellular scaffolds, little consensus exists regarding the quality of the decellularized extracellular matrix (ECM). Metrics used to evaluate ECM performance are study-specific, subjective, and semi-quantitative. Thus, we focused on developing computational methods to examine corneal decellularization efficacy.

METHODS: Corneal decellularization was performed using 1% and 4% zwitterionic surfactant concentrations for 2- and 4-day periods. Conventional biochemical and blinded semi-quantitative histological assessments were compared with automated scaffold evaluations based on textual analyses of digital micrographs to examine decellularization efficacy. The gray level co-occurrence matrix (GLCM), in combination with discrete wavelet transform (DWT) analysis, generated textual parameters that were used as input data to create contemporary artificial intelligence (AI) models based on random forests and vector machine algorithms.

RESULTS: Each decellularization approach supported effective DNA removal (>95%), while manual scoring of treatment groups revealed statistically significant differences in DNA removal and changes to collagen fiber structure post-decellularization. GLCM and DWT analyses illustrated significant variations in textual parameters between the native and decellularized stroma. Regarding the machine learning models, we obtained the best performance using inverse difference moment, GLCM contrast, GLCM correlation, angular second moment and homogeneity, and wavelet coefficient energies for input data. Likewise, the estimated accuracy of the random forests model was 82.67%, while the area under the receiver operating characteristics curve was approximately 0.92. The classification accuracy of the support vector machine was 80.5% which was relatively similar to random forests. However, the support vector approach had significantly lower discriminatory power in separating treated stromal ROIs from intact stromal ROIs with the area under the receiver operating characteristics curve of 0.84.

DISCUSSION & CONCLUSIONS: From our investigations, we realized that exposing corneal stromal tissues to low surfactant concentrations leads to significant changes in the features of GLCM and DWT, as with conventional histological scoring. These changes indicate a substantial increase in textural uniformity and local homogeneity and a reduction of stromal co-occurrence and wavelet

heterogeneity, probably due to the loss of keratocytes and their nuclei. In this study, we also demonstrate that it is possible to develop and test machine learning models based on support vector machines and random forests using GLCM and DWT as input data, which will substantially decrease evaluation time and complexity as evidenced using multiple scorers that each required at least two days to perform these assessments.

Keywords: Eye, Decellularised matrices

PP-310

Biological and mechanical characterization of a decellularized porcine esophageal matrix

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INTRODUCTION: The restoration of digestive continuity after the ablation of a portion of the esophagus is currently ensured by the interposition of a colon segment or by the tubulation of the stomach. The development of a tissue-engineered substitute made from decellularized esophageal matrix (DM) from pigs is part of the improvement of surgical treatment. It has also been shown that the in-situ tissue integration of biological matrices is optimal when cellularized. Therefore, the objectives of this work were to characterize the biological and mechanical properties of DM and to study the immunoprivileged character of Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) to improve DM integration.

METHODS: Esophagi were collected from pigs and successively perfused with chemical and enzymatic solutions in a bioreactor. The efficacy of the decellularization and the histological structure were analyzed with hematoxylin eosin saffron (HES) staining. Protein content of the extracellular matrix (ECM) was analyzed by mass spectrometry. DMs were evaluated for suture retention strength, tensile failure and burst pressure. To ensure the benefit of WJ-MSCs in our tissue engineering application, human leukocyte antigens of class II (HLA-DR) of WJ-MSCs were immuno-labeled and cells were analyzed by flow cytometry (Canto II Thor). Finally, a co-culture analysis with PBMCs was carried out to determine the activation of T cells when in contact with WJ-MSCs.

RESULTS: No remaining cells were observed in HES staining, and the cohesion of the tissue was preserved after the decellularization. Proteomic analysis suggests the potential of the DM to support a variety of biological activities based on its high complexity with a rich and cross-linked collagenous matrix. DM had excellent suture retention strength in comparison to the native esophagus (NE) (5.3 ± 0.4 N and 2.4 ± 1.3 N, respectively). DM had significantly increased tensile strength in the longitudinal and circumferential fibers orientations in comparison to NE. DM had a burst pressure (726.1 ± 213.8 mmHg) compatible with its implantation as an esophageal substitute. We showed that only 0.3% of WJ-MSCs expressed HLA-DR. This HLA-DR is the cause of acute rejection in allografts. Moreover, in contact with WJ-MSCs, PBMCs poorly expressed CD69 and CD25, both T cell markers.

DISCUSSION & CONCLUSIONS: We produced a collagenic decellularized matrix with suitable mechanical properties for implantation. Moreover, we showed that this DM can be recellularized with low immunogenic WJ-MSCs, thus promoting tissue integration and remodeling once implanted.

Keywords: Biomaterials, Stem cells - general

PP-312

The importance of the choice of dissolution media for monitoring drug release: case study with modified-hyaluronan nanofibers

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INTRODUCTION: The release of active substances (API) from pharmaceutical formulations depends on many factors, including physical-chemical properties of the API, the carrier, and the release medium. In the case of nanofibrous mats, the carrier properties include, e.g., polymer matrix erosion, swelling, and preservation of nanofibrous structure. Release experiments are usually performed in phosphate buffer (PB), which is depleted of biomolecules that naturally occur in the body. Due to the binding ability between transport proteins and API, the release profile of API in the environment containing biomolecules may be different in comparison to PB. This can completely change the release kinetics in real applications.

METHODS: Water stable nanofibrous mats from hydrophobized hyaluronan derivatives were prepared by electrospinning. The mats consisted of lauroyl hyaluronan (L-HA) or a combination of furanyl hyaluronan (F-HA) and L-HA in two different ratios with incorporated octenidine dihydrochloride (OCT) or triclosan (TRI) [1,2]. The API release was studied in PB, PB with bovine serum albumin (PBA, concentration 20 g/L) and porcine plasma (PL) for 48 hours. The methodology for API release and polymer solubilization is described in [3].

RESULTS: The rate of L-HA solubilization was highest in PBA>PL>PB. In comparison, the composites F-HA/L-HA were solubilized faster, their rate of solubilization followed the order PL>PBA>PB. The maximum released amount of TRI to PB, PBA and PL was 20, 70, and 60 wt%, respectively. OCT was not released into PB but was gradually released into PBA and PL, depending on the matrix composition (max. 40 wt% in PBA, 20 wt% in PL).

DISCUSSION & CONCLUSIONS: Swelling and solubilization of the nanofibrous mats and the release of API was greatly influenced by the media. Generally, the release of TRI was governed by diffusion, with higher release rate from F-HA/L-HA mats due to their porous structure and higher solubilization. TRI was released in higher amounts into the media with biomolecules, which can bind TRI. OCT was not released into PB due to its low solubility and negligible matrix solubilization in this medium. The higher release of OCT to PBA and PL from L-HA/F-HA was mostly governed by the solubilization of polymer matrix. Since the difference between API release into PB and media containing biomolecules could be vast, it should be more common to assess the release of API from pharmaceutical formulations into media similar to the environment in intended applications.

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Keywords: Drug delivery, Biomaterials

PP-313

Mucoadhesive nanomaterials – Composition and size matters

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INTRODUCTION: Nano-based drug dosage forms have attracted attention of pharmaceutical industry due to their unique properties and ability to overcome various physiological barriers and surfaces. Among all, the mucosal surface plays a crucial role for in situ sublingual, eye, vaginal and intestinal delivery of large number of drugs. Administration through these tissues is a challenge due to the poor mucoadhesion of the current formulations often leading to very low DDS stability and therefore insufficient bioavailability of delivered drug. For example, 90% of the drugs used in ophthalmology are administered with ocular drops onto the mucosal layer of the cornea, due to poor mucoadhesion of DDS formulation and only 0.0006% to 0.02% of the active molecules administered onto the cornea are able to reach the anterior chamber of the eye, where most of intended drug targets are located [1]. From the nutrition point of view, interactions with intestinal mucosal barrier are crucial for biomolecules reception. The development of efficient mucoadhesive drug delivery systems (DDS) is thus a crucial task to improve bioavailability, stability and therapeutic performance of drugs delivered in situ on various mucosal tissues. In nanomaterials, the degree of mucoadhesion depends on several aspects – dimension, chemical composition, surface charge and wettability.

METHODS: In our work, several types of nanomaterials in form of nanoparticles and nanofibres based on PCL, chitosan, silica and CeO₂ were studied on mucoadhesion in vitro. Size and surface morphology was evaluated using SEM and DLS. The material-mucin interactions were tested by several methods under simulated physiological conditions. Moreover, concentration dependent cytotoxicity of nanomaterials to intestinal epithelium model based on Caco-2 cells and corneal epithelium model based on HCE cells was studied in vitro.

RESULTS: The nanomaterial-mucin interactions were proven to be composition and size dependent with the best results obtained from chitosan nanomaterials. In the case of PCL nanofibres, strong impact of fibre morphology was observed with amount of bound mucin growing with decreasing fibre diameter.

DISCUSSION & CONCLUSIONS: Increasing specific surface leads to improved mucin adhesion to nanomaterials. Chitosan nanomaterials represent highly mucoadhesive DDS due to their positive charge and strong interactions with mucin.

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Keywords: Nanomaterials (inc graphene), Drug delivery

PP-314

Defined Nanofiber Scaffolds Modulate Human Gingival Keratinocyte Behavior

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INTRODUCTION: Biophysical stimuli, like extracellular environment and the stiffness and strain derived from it, modulate cellular functions, for example morphology and differentiation. For biomaterial-based tissue regeneration, defined nanofiber structures and stiffness of electrospun three-dimensional scaffolds are increasingly coming into focus. In the human oral cavity, the integrity of the oral epithelium is crucial to function as a pathogen barrier. Hence, fast and complete regeneration after wounding is fundamental. In this regard, we used in our study immortalized human gingival keratinocytes (ihGK), to examine cellular functions on electrospun nanofiber scaffolds, differing in thickness and configuration. The investigation of cell morphology, proliferation and differentiation of ihGKs on these defined model surfaces, shall provide deeper insights for tissue regeneration.

METHODS: ihGKs seeded in low density on electrospun nanofiber scaffolds, varying in fiber thickness (600-800 nm, 1.2-1.7 μ m, 2.0-2.5 μ m) and configuration (random and aligned), were examined for their cell shape. Morphometric measurements were performed using ImageJ and eight cell specific parameters were analyzed. Further investigations considering differentiation markers, were performed with digital droplet PCR and statistically evaluated.

RESULTS: Independent of fiber thickness, ihGKs seeded on random nanofiber scaffolds displayed a rounder and more circulated cell shape. Aligned nanofiber scaffolds induced an elongated cell shape with an increase in the major axis. Differences in gene expression were detected independent of fiber orientation and thickness. Among all tested scaffolds, particularly small nanofiber thickness of 600-800 nm led to distinct differentiation patterns in ihGKs, regarding early and terminal differentiation markers. While random-orientated nanofibers triggered a basal to early-differentiated cell behavior, aligned nanofiber orientation induced early to terminal differentiation.

DISCUSSION & CONCLUSIONS: According to our data, nanofiber orientation has a great impact on ihGK morphology. Independent of the nanofiber thickness, ihGKs seem to adapt with an unusual elongated cell shape to an aligned fiber environment. Especially with the smallest fiber thickness of 600-800 nm, the elongated cell shape on aligned nanofibers correlates with a progressive differentiated cell behavior compared to ihGKs cultivated on random orientated nanofibers. In the oral gingival epithelium cell flattening correlates with terminal differentiation in the uppermost cell layers. Our findings on ihGKs suggest that ihGK cell behavior, in our case morphology and

differentiation, is modulated as function of scaffold configuration. With further investigations of differentiation markers, proliferation and cytoskeletal changes, biomaterial parameters could be used as read-out, to identify distinct nanofiber-based scaffold configurations for prospective strategies in tissue regeneration.

Keywords: Biomaterials, Differentiation

PP-315

***In vitro* cytotoxicity of metallic nanoparticles towards macrophages**

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INTRODUCTION: After an injury, a complex and dynamic biological process termed wound healing is initiated to restore the damaged tissues. Macrophages play a central role in this process: during the inflammatory phase, M1 macrophages clear the debris and apoptotic cells by phagocytosis and secrete pro-inflammatory cytokines; during the proliferation and remodelling phase, M2 macrophages secrete growth factors that stimulate the migration and proliferation of fibroblasts and endothelial cells (Kloc et al., 2019).

The extraordinary health benefits of antibiotics, used to treat and prevent infection, are under threat due to the rapid emergence of antibiotic resistant bacteria. Therefore, the use of non-antibiotic antimicrobials, such as metallic nanoparticles (NPs), against which bacteria have not been seen to develop resistance, is preferable (Sánchez-López et al., 2020).

Being macrophages the cells most exposed to external agents, including metallic NPs, it is crucial to understand the cytotoxic effects of those NPs towards macrophages. Thus, in this work different metallic NPs were produced, characterized and their cytotoxicity evaluated using macrophages and epithelial cells.

METHODS: Silver, gold, zinc and copper NPs were synthesized by chemical precipitation. The NPs were characterized by dynamic light scattering (DLS), scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) and UV-Vis spectroscopy. For cytotoxicity evaluation, the NPs were added to macrophage cultures, differentiated from the THP-1 monocyte cell line using 8 nM phorbol 12-myristate 13-acetate (PMA), and cell viability was determined using the resazurin assay. The cytotoxicity of NPs to Vero cells (cell line recommended by the ISO-10993 standard to perform cytotoxicity experiments) was also determined.

RESULTS: The gold and silver NPs are the ones with smaller diameter (61 ± 4 nm and polydispersity index of 0.2) and the ones less toxic to macrophages ($IC_{50} < 50$ μ g/mL). The copper NPs are the ones that cause the highest macrophage death (IC_{50} of 6.25 μ g/mL). For the same NPs concentration, the IC_{50} values are superior in Vero cells regarding to macrophages.

DISCUSSION & CONCLUSIONS: The safety limit of different NPs was determined for macrophages and Vero cells, allowing their integration in fibrous mats to create an antimicrobial wound dressing.

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Keywords: Immunity / immunomodulation / macrophage, Wound healing

PP-317

Enhanced skeletal muscle cell response on dynamically stimulated electroactive scaffolds

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INTRODUCTION: Piezoelectric biomaterials have attracted interest offering new opportunities for skeletal muscle tissue engineering (TE) [1], allowing improved biomimetic TE approach. In fact, electroactive stimuli have demonstrated to be an important parameter to be considered for TE. However, their effect at the cellular level [2] is still poorly applied and understood in muscle regeneration. So, this work reports on active biomaterials with and without magnetic nanoparticles suitable for effective myoblasts differentiation in a biomimetic microenvironment providing the electro-mechanical stimuli associated to this tissue in the human body and their effect on proteomic analysis.

METHODS: Piezoelectric and magnetoelectric films were obtained by solvent casting. Poly(vinylidene fluoride) (PVDF) is the piezoelectric material used and the magnetoelectric films are the combination of a piezoelectric polymer, poly(vinylidene fluoride-trifluoro-ethylene) (P(VDF-TrFE)), and magnetostrictive particles (CoFe₂O₄).

Non-poled and poled (with negative and positive surface charge) piezoelectric and magnetoelectric composites were used to investigate their effect on C2C12 myoblast differentiation. The nanocomposites were fully characterized, and cell cultures were also performed under static and dynamic conditions through the use of two types of bioreactors (magnetic and mechanical). The proteome analysis of C2C12 cell differentiation under static conditions was also analysed by label-free liquid chromatography tandem mass spectrometry (LC-MS/MS).

RESULTS: The results demonstrated that the biomaterial surface had influence on the C2C12 differentiation. The proteome analysis demonstrated, after 5days of differentiation, that the number of significantly expressed proteins were 23 and 31 (such as Ckm, Tmem14c, Serpinb6a, adh7 and Car3) in cells grown on negative and positive surface charge, respectively. Before cell culture assays, the stimuli provided by the produced biomaterials was quantified, obtaining mechanical (0.013µm.mm²) and electrical (up to 64µV) stimulation.

Regarding the stimulation by magnetic bioreactor, it was demonstrated that after 5days of differentiation, the fusion and maturation index values were more significative in charged samples. Non-poled samples lead to higher myotubes diameters and poled samples to higher myotubes length. Also, the poled samples present higher creatine kinase values compared with the non-poled ones, under dynamic conditions.

DISCUSSION & CONCLUSIONS: This work demonstrates the relevance of electromechanically active microenvironments for muscle TE. Both positive and negative surface charge promotes proteins related with the contraction of the skeletal muscle cells, with a more pronounced effect with the negative charged surfaces.

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Keywords: Electroactive materials, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-319

Electrically Conductive Biomaterials for the Preservation and Regeneration of Soft/Hard Tissue

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INTRODUCTION: Combat trauma results in severe, complex injuries involving multiple tissue types. There is growing evidence that an electrical stimulus applied to a wound can enhance tissue regeneration. Therefore, in early wound management it is important to optimise endogenous responses including the bioelectric current.

METHODS: In order to fabricate electroactive wound dressings, we have incorporated a network of the conductive polymer polypyrrole (Ppy) into non-biodegradable wound care products. We are also developing electrically controlled delivery methods using conductive polymers incorporated in the wound dressings for therapeutic use that enhance tissue preservation. In parallel we are investigating the migratory and proliferative properties of skin cell types and their response to electrical stimulation.

RESULTS: Comparison of pristine wound dressings to Ppy coated wound dressing shows that foam, alginate and aquafiber based dressings are electrically conductive. Preliminary data shows that drug can be electrochemically loaded into the wound dressings and released upon electrical stimulation. We have also developed an electrical stimulation paradigm by performing wound healing experiments on indium tin oxide coated glass and applying an electrical stimulus. Fibroblasts demonstrate enhanced wound healing at 50mV/mm DC electrical stimulation.

DISCUSSION & CONCLUSIONS: The conductive wound dressings will allow direct electrical stimulation of the wound to achieve pro-regenerative activity. Combined with a drug delivery system, this will allow localised drug release to the wound to stimulate wound healing. The wound dressings will also provide a direct electrical stimulation to the wound. Overall, we aim to aid the healing of complex wounds by enhancing the endogenous regenerative response of cells in the skin and therefore decrease the recovery period.

Keywords: Wound healing, Drug delivery

PP-320

Electrically conductive hydrogel loaded with PEDOT-Kartogenin nanoparticles for articular cartilage regeneration

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INTRODUCTION: Articular cartilage defects resultant from trauma or highly prevalent degenerative diseases (e.g., osteoarthritis) fail to self-regenerate due to low chondrocyte density and avascular nature of cartilage. Cartilage tissue engineering (CTE) combining stem cells, biomaterials and bioactive factors emerged as a promising alternative to the current ineffective treatments. Hydrogels are suitable candidates for CTE due to their ability to recapitulate the biophysical properties of native cartilage. Moreover, hydrogels can be combined with conductive materials to mimic the natural electrochemical features of the tissue while also allowing controlled drug delivery through external electrical stimulation (ES) [1]. In this work, we present a novel electrically conductive hydrogel loaded with a chondroinductive drug (Kartogenin, KTG), which can be released in a controlled manner upon ES and promote the chondrogenic differentiation of human mesenchymal stem/stromal cells (hMSCs).

METHODS: PEDOT-KTG nanoparticles (NPs) were fabricated through in situ emulsion polymerization based in previous protocols [2], and characterized by SEM, TEM, AFM, DLS, FTIR and cyclic voltammetry. PEDOT-KTG NPs were loaded in a 3D hyaluronic acid (HA)-chondroitin sulfate (CS)-PEGDA hydrogel and the composite was characterized in terms of its structural/physical properties. KTG release was studied upon ES by HPLC analysis. The biocompatibility and chondrogenic potential of these NPs were assessed in vitro using 2D hMSC cultures with and without ES. hMSCs were then encapsulated in HA-CS-PEGDA-PEDOT-KTG hydrogel and exposed to ES every 3 days for 21 days. The final tissue constructs were evaluated in terms of cell viability, GAG production and cartilage-marker gene expression (RT-qPCR).

RESULTS: KTG was incorporated in PEDOT NPs with a drug loading efficiency of 60%, originating electroactive PEDOT-KTG NPs with a diameter of 172 ± 90 nm and a zeta-potential of -75 mV. KTG release was shown to be highly controllable through ES. When combined with 2D cultures of hMSCs under ES, PEDOT-KTG NPs demonstrated high biocompatibility and capacity to enhance GAG production. hMSCs encapsulated in HA-CS-PEGDA-PEDOT-KTG hydrogels and cultured for 21 days under ES showed high cell viability and upregulation of chondrogenic marker genes.

DISCUSSION & CONCLUSIONS: The HA-CS-PEGDA-PEDOT-KTG hydrogel system was able to promote the controlled release of KTG upon ES and consequently support the chondrogenic differentiation of hMSCs. Overall, our results highlight the potential of combining biomimetic electrically conductive hydrogels and ES towards improved CTE strategies.

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Keywords: Cartilage / joint and arthritic conditions, Hydrogels and injectable systems

PP-321

Characterisation of Poly(3-hydroxybutyrate)/Graphene oxide (P(3HB)/GO) composite films for bone tissue engineering

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INTRODUCTION: *Burkholderia sp.* was found to produce polyhydroxyalkanoates (PHAs) using different of carbon sources such as fatty acids and sucrose. Recently, PHAs have been used as biological materials because of their characteristics such as biodegradability, biocompatibility and sustainable materials. Moreover, GO has been used to improve the properties of the material such as the mechanical properties and cell viability. This study aims to develop the scaffolds using PHAs and its composites to achieve suitable mechanical, physical and chemical properties required for bone tissue engineering.

METHODS: Neat P(3HB) and P(3HB)/GO films were prepared using solvent casting method. P(3HB)/GO composites were prepared in proportions of 1, 2, 5 and 10% w/w and aged for 1-month prior to further characterisation. Chemical properties were determined using Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR). Thermal properties were also determined using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Mechanical tensile testing was used to verify the properties of neat P(3HB) and P(3HB)/GO composite films. Finally, the molecular weight of the polymer was obtained using gel permeation chromatography (GPC).

RESULTS: Chemical analysis confirmed that the chemical structure of the polymers obtained using *Burkholderia sp.* fermentation was indeed P(3HB). The P(3HB) exhibited the weight-average molecular weight (M_w) of 290,116 Da, number-average molecular weight (M_n) of 122,052 Da and polydispersity index (PDI) of 2.38. Thermal analysis of neat P(3HB) demonstrated a glass transition temperature (T_g) at 4.72 °C, crystallization temperature (T_c) at 51.73 °C, melting temperature (T_m) at 174.71 °C and the degradation temperature (T_d) at 290.97 °C. The addition of GO in P(3HB) affected the T_g and T_c which were not observed in the composite films. However, there were no significant differences when comparing T_m and T_d of the neat P(3HB), which were around 172-174 °C and 290-300 °C respectively. The wt% of GO in P(3HB) also affected the mechanical properties of the composite films, leading to tailorable mechanical properties. The mechanical properties of P(3HB)/1%GO aged 1-month film exhibited the highest Young's modulus which was around 1300 MPa, suitable for cortical bone repair.

DISCUSSION & CONCLUSIONS: In this study we studied P(3HB) production and an in-depth characterisation of neat P(3HB) and P(3HB)/GO composite films to be used in the bone tissue engineering applications. The properties were found to be suitable for various processing techniques and could lead to bespoke mechanical properties depending on the filler concentration. In future these composites will be tested for their biocompatibility and antimicrobial properties.

Keywords: Biomaterials,

PP-322

Nano black phosphorus/graphene oxide functionalized collagen scaffold with enhanced photo-thermal and biomimetic mineralization for in situ infectious bone repair

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INTRODUCTION: Infective bone defect is a common clinical problem. Infection control and bone defect reconstruction are the two main missions in traditional standard treatments. Combining the systemic application of antibiotics with autograft or allograft have been widely used in bacterial infectious bone defects. However, these treatments have several shortcomings, such as multidrug-resistant bacteria, donor site complications, and immune rejection. Therefore, developing multifunctional bone graft substitutes with favorable antibacterial activity, osteoconductivity, and osteoinductivity would be a treatment strategy of great clinical significance. Collagen (Col) has been widely used to develop bone repair materials due to its good biocompatibility and biodegradability. However, collagen-based materials have the disadvantages of insufficient antibacterial performance and poor osteogenic activity. Graphene oxide (GO) and black phosphorus (BP) are commonly used two-dimensional inorganic materials, which have good photo-thermal anti-bactericidal effects under near-infrared (NIR) light. At the same time, NIR light can promote degradation of BP into PO₄³⁻, which accelerates the biomineralization process and promotes osteoconductivity. Therefore, the preparation of GO/BP functionalized Col scaffold with photo-thermal antibacterial activity and in situ biomineralization ability may provide new insights into developing multifunctional bone graft substitute for the repair of infectious bone defects.

METHODS: GO/BP nanoparticles were prepared and incorporated into Col scaffolds by one-step method. The degradation of BP under 808nm NIR was characterized. Subsequently, the physical and chemical properties of Col-GO-BP were characterized. Moreover, the biomimetic mineralization ability, photo-thermal antibacterial performance, and cell viability were evaluated. Finally, the infectious skull defect of the rat was used to evaluate their anti-infection and bone regeneration abilities.

RESULTS: GO/BP nanoparticles were successfully prepared with lamellar structure under TEM. Under the 808nm NIR irradiation, BP gradually degraded into PO₄³⁻. The temperature of the prepared Col-GO-BP scaffold could stably be elevated. The cell experiments in vitro showed that the Col-GO-BP scaffold could effectively adsorb cells, promote cell proliferation, and enhance osteoblastic differentiation. The results of animal experiments in vivo showed that the Col-GO-BP scaffold could effectively promote the repair of infectious bone defects.

DISCUSSION & CONCLUSIONS: We successfully developed GO/BP functionalized Col scaffold. The GO incorporation improved cell adhesion, proliferation, and osteogenic differentiation. Moreover, the BP not only provided a phosphorus source and nucleation sites, but also accelerated the reaction between PO₄³⁻ and Ca²⁺ to promote biomineralization. Finally, the fabricated scaffold showed a good bone regeneration effect in infective bone defects by killing the bacteria, regulating the local inflammation, and improving the oxidative stress.

Keywords: Bone and bone disorders (osteoporosis etc), Composite materials

PP-323

Bacterial Cellulose and Its Blends for Bone Tissue Engineering

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INTRODUCTION: Bacterial cellulose (BC), a natural polymer has low biotoxicity, strong hydrophilicity, good cell adherence and relatively simple production methodology in comparison to some other natural materials. Hence, BC has great potential as a tissue engineering material. Also, BC is one of the stiffest materials, hence it is highly suitable for bone tissue engineering. This work focuses on BC and blends of BC with polyhydroxyalkanoates (PHA), a natural polyester of bacterial origin

METHODS: The production and purification of BC:

BC was produced using *G.xylinus*. The bacteria were grown in HS media under static conditions at 30 °C. The wet pellicles are harvested from the upper surface of the inoculum, washed in 0.1 M NaOH and deionized water. Dry BC pellicles were obtained via air drying and freeze drying.

The blend of BC and Poly(3-hydroxyoctanoate-co-3-hydroxydecanoate), P(3HO-co-3HD):

The wet BC pellicles were soaked in acetone for dehydration, then transferred to 5% P(3HO-co-3HD) solution in acetone overnight. The pellicles were dried until the acetone evaporates.

RESULTS: The wet BC pellicles were composed of unaligned nanofibers, as observed via SEM and freeze-dried samples exhibited uneven porosity. BC exhibited a melting point between 50-100 °C and the degradation was found to start at around 300 °C.

In comparison to pure BC pellicles, P(3HO-co-3HD) blend pellicles were thicker in appearance and quite elastomeric. FTIR results clearly showed the P(3HO-co-3HD)-specific methyl group and carbonyl group linked to an ester group. The mechanical properties of neat BC were compared to that of blend and was found to be distinctly different.

DISCUSSION & CONCLUSIONS: Both the melting point and the degradation temperature of BC are higher than the normal human body temperature (37 °C), hence it will be expected to be thermally stable in the human body. Neat BC was relatively brittle and stiff as compared to the blend with P(3HO-co-3HD), both highly useful in the context of bone tissue engineering.

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Keywords: Biomaterials,

PP-324

Magnesium Doped Bioactive Glass: an alternative to Copper Doped Bioactive Glass regarding Antibacterial properties

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INTRODUCTION: The use of synthetic implants for bone restoration is associated with high risk of bacterial colonization, resulting in implant failure. Bioactive glasses (BG) are among the most used materials in bone regeneration applications. However, this material is not effective in maintaining an infection-free wound. One of the most used BG dopants with antibacterial properties is Copper (Cu). However, Cu is highly cytotoxic, which is detrimental for tissue regeneration applications.[1] To solve this problem, several ions have been studied as a substitute to Cu, such as Magnesium (Mg), due to its antibacterial properties and biocompatibility. This work aims to improve the performance of BG-coated implants by doping BG powders with Cu and Mg and comparing their anti-microbiological activity against several microorganisms.

METHODS: The 45S5 Bioglass™ was doped with Cu or Mg at different concentrations (1, 2 and 4mol%). The BGs were prepared by the melt quenching technique, and then characterized both physically and biologically. Biological analysis focuses on cell responses and microbiological assays. Cell responses were analyzed by cytotoxicity assays, using Saos-2 cells. The microbiological assays allow Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination using both Gram-negative (*E.coli*) and Gram-positive bacteria (*methicillin-resistant Staphylococcus aureus* (MRSA) and *S.mutans*).

RESULTS: The integration of the above therapeutic ions does not show a significant change in the glass structure. Cell responses show no alteration of cell viability for Mg doped samples, with viable concentrations of 25mg/ml. Although, Cu doped samples presented an increase in cytotoxicity with the increase in doping concentration. Additionally, the incorporation of Mg in BG led to MICs comparable to when Cu-doped BGs were used. MRSA was successfully inhibited by both materials using 50mg/ml, the *E.coli* only needed 25mg/ml and the *S.mutans* was completely inhibited by using, any of the doped Cu samples and 1mol% Mg doped BG at 25mg/ml. However, the MBC analysis only presented the same results between Cu-doped BGs and Mg-doped BGs when used against *E.coli*.

DISCUSSION & CONCLUSIONS: These results show that Mg-doped BG is a promising material for preventing bacterial infections without compromising cell responses.

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Keywords: Ceramics, Infection

PP-325

Synthesis, characterization and processing as a fibrous scaffold of highly-bioadhesive polyurethane urea inspired by mussels

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INTRODUCTION: The need is growing to obtain solid adhesive scaffolds that can be processed to create friendly micro-environments, directing cellular behaviours and tissue regeneration. A facile method was used to incorporate a mussel-inspired adhesive moiety, dopamine, into segmented polyurethanes, based on PCL-PEG-PCL copolymers. Dopamine was chemically bonded with lysine and used as a chain extender to obtain a solid biodegradable elastomer capable of strongly adhering to different materials after melting occurs.

METHODS: The polyurethane was synthesized through a step-growth polymerization based on 1,4-butanediol (BDI) as the hard segment, triblock copolymers as soft segments, and lysine-dopamine as the chain extender. The triblock copolymer PCL-PEG-PCL was synthesized by Ring-Opening Polymerization. Lysine-dopamine (LDA) was synthesized from L-Lysine and dopamine-HCl as reported in literature.

The product was characterized by FTIR, HNMR, SEC, DSC and UV-Vis confirming the success of the synthesis reactions. A complete chemical-physical characterization was performed, and adhesion strength was evaluated by lap shear tests. Lysine alone was used as a chain extender to produce a similar polyurethane control group.

The synthesized polyurethanes were then dispersed in hexafluoroisopropanol and processed through electrospinning to obtain a random nanofibrous three-dimensional scaffold and through 3D printing to obtain oriented micrometre fibres. SEM was adopted as an investigation method to study the morphology of the fibres.

RESULTS: The DSC analysis showed two different melting temperatures inside the polyurethane. The first one at 38°C is correlated to the PEG block and the other at 56°C is due to the PCL. The low melting point (55°C), combined with the observed adhesiveness after melting, opens multiple possible applications for this novel polyurethane.

The interaction with a high-energy surface (glass and metal) at room temperature was remarkable (2.6 MPa). The adhesion was also evaluated with porcine skin underwater at 37°C resulting in 30kPa. The degradation test underlines the remarkable hydrolytic resistance of the synthesized PU which is a fundamental design requirement for tissue-engineered scaffolds to maintain structure and function over time.

DISCUSSION & CONCLUSIONS: We have synthesized, characterized, and processed a novel biodegradable segmented polyurethane as biomaterial. It can be processed to generate scaffolds with fibres ranging from nano to micrometres, and thanks to LDA used as a dopamine-containing chain extender, to produce biodegradable adhesives and to print adhesive micropatterns on several surfaces. This polyurethane has a remarkable hydrolytic resistance to degradation, a low melting point (55°C), low swelling and high adhesion after melting.

We are currently working on electroactive components integration inside the fibrous scaffolds.

Keywords: Polymers - natural / synthetic / responsive, Biomaterials

PP-326

Adhesive and biodegradable membranes from marine origin raw materials for soft tissue engineering applications

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INTRODUCTION: Tissue engineering (TE) is a versatile solution for re-establishment of normal biological functions by using bioactive materials to fill the injury site and thus, provide mechanical support and cell adhesive platform. Natural materials have been proposed for TE applications, like chitosan - a polysaccharide obtained by the deacetylation of chitin, a structural element of the crustaceans exoskeleton. Chitosan is often combined with collagen to improve its mechanical properties and cytocompatibility. Collagen type I (Coll I) can be obtained from fish by-products, representing a sustainable strategy for marine by-products valorization with economic and ecological advantages¹. Herein, we obtained a new adhesive by conjugating marine Coll I with a catechol derivative 3,4-dihydroxybenzaldehyde(3,4-DB)². The conjugate (Coll-Cat) was blended with chitosan (ChitColl-Cat) and evaluated as a material for regenerating soft tissues.

METHODS: Coll I was extracted from Atlantic cod (*Gadus morhua*) skins by supercritical fluids technology using CO₂ acidified water. Schiff's base reaction was performed by stirring 46mg/mL of 3,4-DB dissolved in osmotized water at 60°C and 5mg/mL of collagen dissolved in acetic acid (AcOH) for 2h at RT. The modification was confirmed by UV spectrophotometry and ¹H-NMR. Membranes prepared from 2.5% w/v of deacetylated chitosan in AcOH (1.5% v/v) and glycerol (5% v/v) were used as controls and 1.5% w/v of chitosan solution to which was added either Coll I (1% w/v) or Coll-Cat (1% w/v) were used to obtain a ChitColl or a ChitColl-Cat blend, respectively. The membranes were processed by spin-coating (200rpm,3min) in a Petri dish (Ø90 mm) and then dried at 37°C.

RESULTS: The ChitColl-Cat membranes presented a dark brown color due to the presence of catechol groups, different from the chitosan (transparent) and ChitColl (opaque) membranes. The functionalization with catechol groups enhanced the adhesion strength (22.5±4.9kPa), which is similar to the natural adhesives used in the clinical context and higher than some synthetic adhesives in wet environments. Preliminary biological tests were performed by seeding fibroblastic cells on the surface of the membranes, which showed an improved cell attachment and viability for the biomimetic membranes.

DISCUSSION & CONCLUSIONS: The sustainable use of biomimetic approaches and the under-exploited marine-derived polymers to develop new adhesive membranes demonstrated to be highly promising to adhere closely to the tissue and provide mechanical and structural support for an effective soft tissue repair.

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Keywords: Biomaterials,

PP-327

Continuous Gradients Forming Bioprinted Devices: 3D, High Throughput, and Environmentally Conscient Precision Medicine

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INTRODUCTION: In vitro modeling is being put forward as a replacement for animal models in widespread drug testing by 2030 by many international institutions[1], [2]. However, in vitro protocols for drug titration still rely on 2-dimensional (2D) cell culture, with discrete concentrations in well-plates, one per each well, having to interpolate data between them. This process requires heavy and specific machinery, high volume data collection, and amount of plastic waste, only to come short on representativity of the tissue.

METHODS: For this reason, we propose a biodegradable bioprinted microfluidic device, with no requirement of plastic support, for 3D, continuous drug titration. Designed to form a linear gradient in a microfluidic channel between two reservoirs, the cellular effect observed in the channels' walls can be traced to a concentration in solution with precision.

RESULTS: The gradient's linearity for small and large molecules was verified, using fluorescein isothiocyanate (FITC) and rhodamine-labelled 70 kDa dextran particles as models for such molecules, respectively. The predictor effect of the platform was tested using SH-SY5Y neuronal differentiation once exposed to retinoic acid. Monitoring proliferation and control markers, an optimal concentration was calculated and tested using inter-spheroid contact.

DISCUSSION & CONCLUSIONS: The platform predicted an optimal concentration for 3D neuronal differentiation, already validated. This way, the platform proved to be conceptually robust, exhibiting reproducibility as an high precision optimization tool.

The bioprinted microfluidic platform showed potential as a next-generation precision medicine tool.

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Keywords: 3D printing and bioprinting, Personalised medicine

PP-328

Soft enzymatic-crosslinked silk fibroin hydrogel lab-on-a-chip for colorectal tumor model: a proof-of-concept

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INTRODUCTION: The gap between animals and humans, the inconsistency between flat in vitro systems and the human body have created the need for solutions for increasing demand for safety screening and speed of new potential pharmaceuticals. Research has increasingly moved towards the design and implementation of microfluidics to tackle the aforementioned problems. Traditionally made out of hard PDMS, softer biodegradable materials based on natural hydrogels are an excellent alternative to building soft microfluidic platforms. Herein, we describe a new approach to fabricate a novel soft cell-laden microfluidic device making use of enzymatically-crosslinked silk fibroin (eSF) hydrogels for applications as a flexible lab-on-a-chip colorectal tumor model.

METHODS: Based on our group's patented "methods of production of eSF hydrogel microfluidic platform", we used standard UV-photolithography and double replica molding to obtain the soft eSF microfluidic platform. The design comprises channels mimicking the tortuous morphology of microvasculature. The eSF hydrogels were prepared as reported previously (1). After physicochemical characterization, HCT-116 colorectal cells were encapsulated in the eSF and endothelial colonic cells seeded in the serpentine microchannel of the microfluidic device. ATP quantification and Alamar blue were performed before and after the perfusion of 5 μ L of Gemcitabine (GEM).

RESULTS: 14% eSF hydrogels works better in terms of stability, flexibility and transparency. SEM images demonstrated that the eSF hydrogel maintained the microfluidic features with excellent fidelity after the entire fabrication process and crosslinking. We next investigated the deformation capacity by performing tensile tests. A mean tensile strain of 103.96 % was found. Alamar blue and ATP quantification of cells showed that HCT-116 cells are viable overall, and the endothelial cells formed a lumen-like structure in the serpentine microchannel. On the other side, perfusion with GEM showed a limited cell viability after day 3.

DISCUSSION & CONCLUSIONS: We aimed at building a simplistic colorectal tumor model. After developing the methodology for obtaining the platform, concentrations were optimized. The 14% eSF showed the best mechanical characteristics, being malleable, stretchable and transparent. Cell assays without any chemotherapeutic remained viable in the proposed design, showing that 14 wt% eSF microfluidic platform has structural stability, the ability to perfuse fluids, and feed the system with nutrients/O₂ while displaying in vivo-like biological responses. The anti-neoplastic effect was only observed after 7 days, as corroborated by other studies.

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Keywords: Biofabrication, Cancer

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Manufacturing of multifunctional nanocarriers and their influence on osteosarcoma spheroids-based in vitro model

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INTRODUCTION: Osteosarcoma is one of the most common primary malignant bone tumor in children and adolescents, occurring commonly from the mesenchymal tissue in the distal femur. It is one of the most aggressive tumor, metastasizing mainly to the lung. Current treatment, that are surgery combined with neoadjuvant chemotherapy and radiotherapy, are often not satisfactory because surgery is not able to successfully control metastasis and chemotherapy leads to drug resistance and side effects, such as nephrotoxicity and cardiotoxicity. For these reasons it is imminently important to look for new treatment technologies. The use of nanoparticles for gene and drugs delivery was explored on osteosarcoma (OS) spheroids-based in vitro models.

METHODS: The nanoparticles (NPs) were composed of chitosan and PLGA and loaded with miRNA-34 - a short RNA structure that has a proven ability of act as tumor suppressor gene, inducing cell cycle arrest and apoptosis in OS cells. Considering the aspect that the co-delivery of multiple drugs maximized the effectiveness of treating OS, reducing systemic toxicity and drug resistance, three other classes of manufactured NPs were realized adding Doxorubicin and Resveratrol to the previous CH-PLGA-miRNA34 core and exploring Layer-by-Layer (LbL) technique, creating a bilayer-nanocoating, by means of pectin and chitosan as polyelectrolytes.

RESULTS: The resulting NPs showed an average diameter ranging from 159 to 242 nm after the LbL functionalisation as confirmed by DLS and TEM. MiRNA-34 and Doxorubicin were successfully entrapment in the core and in the nanolayers with an entrapment efficiency of 81.2% and 62.6% respectively. The resveratrol exhibited the lower entrapment efficiency (10.3%). For the in vitro release studies, 70% of the total payload cargo was released within 2 days. U2OS and SAOS-2 sarcospheres treated with the functionalised NPs showed reduced cell viability, suggesting that intracellular restoration of miRNA-34a and delivery of Doxorubicin could have led to the tumoral cells apoptosis, with remarkable change of morphology as the time of incubation increased.

DISCUSSION & CONCLUSIONS: Nanoparticles with natural nanocoating materials achieved by LbL technique might be more efficient in carrying miR-34a, Doxorubicin, and Resveratrol internal to the core without excessive dispersion of them out of the target site. The NPs showed suitable sustained releases of miRNA and drugs, with a very reduced burst release and a gradual release within the 3 days of incubation.

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Keywords: Drug delivery, Cancer

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Enhancing the Reproducibility of Nanodroplets for Use in Oxygen delivery for Bone Repair

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INTRODUCTION: Hypoxia is associated with many common bone diseases and complications. Perfluorocarbon nanodroplet emulsions offer a potential method for delivery of oxygen to hypoxic tissues due to their high oxygen solubility and small size. Although sonication is the most common laboratory process for making nanodroplets with perfluorocarbons heavier than perfluorobutane, sonication is a very stochastic process and this can result in reduced repeatability. In this work, the sonication process was optimised to allow for improved control over the nanodroplet size distribution and increased reproducibility.

METHODS: To make nanodroplets, lipid films of DSPC and PEG-40s at a molar ratio of 9:1 were made by evaporation then rehydrated in PBS at 4 mg/ml. Perfluoropentane (PFP) was then added and nanodroplets formed using a pulsed sonication (2s on, 15s off) on ice. Sonication time (15-120s) and amplitude (20-100%), perfluoropentane concentration (0-10 %vol) and the sonicator tip height (2-12 mm) were varied and the size of the resultant nanodroplets was then assessed using nanoparticle tracking analysis (NTA).

RESULTS: There was an increase in nanodroplet diameter as a function of PFP concentration ($P < 0.0001$). However, there was no significant difference in nanodroplet diameter between nanodroplets made at concentrations below 5 %vol PFP and in the absence of PFP. In general, increasing tip height led to a lower nanodroplet median diameter and standard deviation of the resultant nanodroplets. Interestingly, increasing the tip height also strengthened the correlation between the PFP concentration and nanodroplet diameter. At 5 %vol PFP at a 12 mm tip height, the median size was 156.4 nm and standard deviation 91.73 nm. These were the smallest nanodroplets not detectable under light microscopy that were significantly different from the control. Greater sonication amplitudes led to smaller nanodroplets, whilst amplitudes <40% failed to emulsify the PFP-lipid solution. Longer sonication times initially led to smaller nanodroplets; however, the nanodroplets became larger again after 90s.

DISCUSSION & CONCLUSIONS: All variables investigated had a significant effect on nanodroplet size, the most significant of which was sonication amplitude. For the application of nanodroplets as an oxygen delivery mechanism to bone, 5 %vol PFP with a 12mm tip height, a sonication amplitude of 100% and sonication time of 90s was considered to be optimal due to the small diameter, small size dispersity and improved reproducibility of the resultant nanodroplets.

Keywords: Nanomaterials (inc graphene),

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Development and characterization of curcumin-loaded Food Grade secondary oil-in-water nanoemulsions and in vitro validation of digestion and muco-adhesion properties through INFOGEST protocol and gut-on-chip technology

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INTRODUCTION: Among oral delivery systems, oil in water nano-emulsions (O/W NEs) are of particular interest to improve pharmacokinetics of lipophilic compounds. Recently, we have implemented a successful strategy to improve O/W NEs stability, based on a polymeric coating on an oil core, through the use of pharma grade formulations. However, in the field of food supplements, food grade materials are the top choice since they combine safety and cost effectiveness. Here, we have replaced pharma grade (PG) with food grade (FG) materials in the preparation of the secondary O/W NEs and performed a comparative study between the two formulations. In order to provide formulations with improved muco-adhesion to the intestinal barrier, secondary O/W NEs were prepared by adding thiol groups to chitosan (Ct) via a simple non-covalent procedure based on N-acetyl-cysteine (NAC) salification, which is compliant with food supplement formulations. PG and FG formulations, in different materials combinations, were prepared and physio-chemically characterized.

METHODS: The emulsions were obtained through the usage of a High-Pressure Homogenizer. The gut-on-chip device were obtained through a PMMA mold and a replica molding by peeling PDMS.

Confocal microscope was used to image cells-emulsion interaction in the gut-on-chip microfluidic device.

INFOGEST protocol was used to assess curcumin bioaccessibility, for formulations and free curcumin.

RESULTS: All formulations were stable in time ($PDI < 0.1$).

The bioaccessibility of curcumin was enhanced for the formulations with respect to free curcumin as demonstrated by INFOGEST protocol assay.

After the treatment with emulsions in the gut-on-chip, we washed the channel to remove the excess of the two systems and analyzed the fluorescence intensity of FITC. We see a low FITC signal of Ct NEs linked to mucus membrane of Caco-2 cells, compared with Ct-NAC NEs, indicating much higher adhesion with respect to Ct- NEs. After washing, the intensity of Ct-NEs signal decreased whereas Ct-NAC showed a greater intensity due to a steady adhesion to the mucus layer.

DISCUSSION & CONCLUSIONS: The obtained findings demonstrate that FG and PG formulations are

similar and have comparable stability. FG formulations (NEs, Ct-NEs and Ct-NAC-NEs) loaded with curcumin conferred an improved bioaccessibility as compared to free curcumin. By comparing muco-adhesion properties of the two polymeric coatings within an intestine on chip device, a significant improvement in the muco-adhesive properties of Ct-NAC was observed with respect to Ct due to the presence of thiol groups. Nonetheless, in-vivo assays are required as a final assessment of the proposed system.

Keywords: Drug delivery, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

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Microfluidic Development of Microparticles

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INTRODUCTION: Patients with type 1 diabetes mellitus can require pancreas transplantation.

However, this can be difficult due to the shortages of whole pancreases. The intrahepatic engraftment of islets can eliminate the need for whole pancreas transplantation. However, poor revascularisation and inflammation results in approximately 60-70% of islet loss. One solution is to deliver molecules to reduce inflammation and improve islet engraftment. Current approaches using double emulsion microparticles with encapsulated molecules have been successful in reducing inflammation. However, fabrication methodologies typically result in polydispersed sized microparticles. We propose a new paradigm of Poly(D,L-Lactide-co-Glycolide) (PLGA)-based microparticles fabricated using microfluidic methods as a reproducible, automated, and scalable process to produce monodispersed microparticles to provide tuneable and controlled release.

METHODS: To generate a water-in-oil-in-water emulsion, the microfluidic technology was used in three different ways. Firstly, a 100µm or 14µm hydrophobic chip with a 100µm hydrophilic chip were used in series. Next, homogenisation of the water-in-oil emulsion was combined with using a 100µm hydrophilic chip. Lastly, an 'in-house' custom made chip was fabricated and used for the one-step generation of microparticles. PLGA was dissolved in either dichloromethane or dimethyl carbonate as the primary oil phase. A solution of human serum albumin was used as the primary aqueous phase. Poly(vinyl alcohol-co-vinyl acetate), formed the secondary aqueous phase.

RESULTS: The use of two microfluidic chips in series resulted in the lowest system stability and variability in encapsulation of the primary droplets. This was caused by inconsistencies in pressure, velocity, and turbulence due to the connections between tubing and chips. The combination of homogenisation with microfluidics resulted in a consistent and reproducible system. Hence, this was 'scaled' up with the Telos® technology where 7 junctions were used to simultaneously generate droplets. Finally, the tailored 'in house' chips allowed tight control in the size and flow of the primary droplets, and hence, the amount of droplet encapsulation at the second junction. This resulted in microparticles that were highly controlled, reproducible, and easily fabricated due to the 'one-step' microfluidic process.

DISCUSSION & CONCLUSIONS: The use of two microfluidic chips in series resulted in the least stable system. The combination of homogenisation with microfluidics was the most stable system, therefore was suitable to scale up with the Telos® technology. The design and fabrication of 'in house' microfluidic devices enabled a tailored double emulsion generation that also has the potential to be scaled up. Lastly, the release properties of the microparticles generated from these systems are undergoing analysis.

Keywords: Biomaterials, Additive manufacturing

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The effects of morphology and composition in electrospun scaffold for vascular tissue engineering applications

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INTRODUCTION: There are currently a variety of vascular tissue engineering approaches used to treat arterial disease [1]. These range from scaffold materials that mimic the native extracellular matrix (ECM) to materials designed to promote angiogenesis [2,3]. However, native autologous vessels used as implants still outperform these novel materials [4]. Therefore, trying to find solutions that promote vascular regeneration leading to fully functional tissues is of crucial importance for the advancement of this field. Herein, we have looked at how the fiber diameter of electrospun polymer scaffolds affects human umbilical vein endothelial cells (ECs) and human umbilical aorta smooth muscle cells (SMCs) performance.

METHODS: Scaffolds were electrospun using varying electrospinning parameters in order to achieve fibers of different diameters. Briefly, 8% and 12% w/v polycaprolactone (PCL) solutions in HFIP; and 14% and 19% w/v PCL solutions in 5:1 chloroform:methanol were electrospun at 250 RPM mandrel speed, resulting in four randomly aligned PCL scaffolds with different fiber diameters: small (S), medium (M), large (L) and extra-large (XL). Scaffold composition was further optimized through the addition of decellularized vascular ECMs. Scaffolds were either seeded with ECs or SMCs. Various quantification methods were performed at time points of 1 day, 6 days and 12 days, including cell viability, RT-qPCR and mechanical analysis.

RESULTS: Fiber diameter and pore size analysis showed that four unique architectures were created, with incrementally increasing diameters/widths ranging from approximately 1.5µm to 5µm. We noted increased cellular infiltration in the largest fibre scaffold. Furthermore, interesting trends in gene expression were noted suggesting that altering the morphology of the scaffold had beneficial effects on the seeded cells. Decellularized ECM was successfully incorporated into the scaffold and confirmed using Fourier Transform Infrared analysis.

DISCUSSION & CONCLUSIONS: This systematic study has shown that cellular performance and gene expression can be modulated through altering the fibre diameter and composition of the scaffold.

We found increase infiltration with the largest fibre, which lead to positive changes in gene

expression. These results highlight the potential of these approaches in vascular tissue engineering.

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Keywords: Biomaterials, Vascular systems / vascularisation and heart

PP-334

From low-viscosity materials to nanocomposite inks: a universal strategy for 3D printing applications

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INTRODUCTION: Conventional bioengineering techniques cannot convey biomaterials with suitable properties for 3D printing of complex tissue structures. Ink engineering is progressively utilized to advance the printability of better therapeutics, with optimized material properties.

This work describes a universal methodology for the development of nanocomposite inks for 3D printing using extremely low-viscous matrices. Our hypothesis relies on the improvement of the interface interaction between the organic and inorganic phases by chemical coupling.

METHODS: To do this, two different matrices were tested and synthesized to appropriate degrees of functionalization: a protein – bovine serum albumin methacrylate (BSAMA) – and a polysaccharide – hyaluronic acid methacrylate (HAMA). Then, as model nanoparticles, bioglass (BGNP) were synthesized and amine-functionalized using a known aminosilane (APTES). In order to create extrudable inks, EDC/NHS chemistry was used to link the amine-modified surface of the BGNP to the inherent carboxylic acids of BSAMA and its amines. Different chemical crosslinking amounts and percentages of BGNP were tested. The reaction was carried overnight and photopolymerization was afterwards performed under visible light, using LAP as a photoinitiator. The modification/grafting of BSAMA, HAMA and BGNP was confirmed and quantified via NMR. Rheological, mechanical and biological behavior of the produced nanocomposites was also evaluated. Simple structures were printed as a proof-of-concept using a bioprinter.

RESULTS: All composite formulations could effectively immobilize and homogeneously disperse the BGNP and turn extremely low viscous materials into shear-thinning formulations, appropriate for 3D printing technologies. The improved interface interactions increased the elastic and viscous modulus of all formulations. More pronounced increases were found with the highest EDC/NHS (4%w/v) and BGNP concentrations (10%w/w). This formulation also displayed the highest Young's modulus of the double-crosslinked construct. Bioactivity in simulated body fluid and cellular assays using adipose-derive stem cells revealed a similar calcium/phosphate ratio to that of hydroxyapatite, and increased viability and metabolic activity on the BGNP-containing formulations, when comparing to pristine scaffolds.

DISCUSSION & CONCLUSIONS: This new-found ink engineering strategy of pre-crosslinking, followed by complete ink reticulation post-print is a must-needed methodology in the field of material sciences, since most natural-based materials lack appropriate rheological properties. Additionally, this system could further reproducibly recapitulate phases of other natures, broadening applicability.

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Keywords: Composite materials, Interfaces - engineered

PP-335

Designing dextran-based inks for visible light-assisted bioprinting of 3D cartilage tissue surrogates

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INTRODUCTION: Cartilage is perhaps the most investigated tissue for bioprinting, largely due to its single cell type and lack of vasculature and innervation. Advanced biological concepts are beginning to be integrated into bioprinting procedures, such as stem cell differentiation protocols and growth factor delivery. These costly processes will require bioactive, easy-to-use hydrogel formulations that do not compromise cell survival.

The range of printable materials with adequate properties for cell encapsulation is limited. Such materials are commonly ionically- or photo-crosslinkable; however, drawbacks include poor crosslinking kinetics, lack of cellular support, and temperature sensitivity. Photocrosslinking of biocompatible hydrogels have focussed on UV-photoinitiators, despite implications on cell viability; these initiators also require more intense light to avoid decreasing crosslinking kinetics.

This work aims to create bioactive, visible light photopolymers for cell encapsulation and cartilage tissue bioprinting. Dextran, functionalised with glycidyl methacrylate (DexGMA), is crosslinked using Eosin-Y, a green light photoinitiator, forming a semi-interpenetrating network with hyaluronan (HA) to mimic the cartilage matrix. We hypothesise DexGMA/HA will offer a user-friendly method for creating viscoelastic hydrogels capable of (1) bioprinting structures via pressure-assisted extrusion and visible light to recapitulate heterogeneous tissues, and (2) supporting encapsulated cells, specifically human pluripotent stem cell-derived chondroprogenitors.

METHODS: Pre-gels, between 5%-10% (w/v), were formulated using varying chain lengths of DexGMA and HA. A photoinitiator system consisting of Eosin-Y, N-vinylcaprolactam and triethanolamine was added to these pre-gels. Immortalised chondrocytes (TC28a2) were encapsulated in pre-gels before reticulating using a hand-made 520 nm LED source for 5-10 minutes. Cell-laden pre-gels were 3D bioprinted using a pressure-assisted extrusion printhead and crosslinked using the 520 nm photocuring toolhead within the Cellink® BioX6.

RESULTS: NMR and FTIR characterisation confirmed GMA substituted onto dextran at a degree of 42% to 54%. Full reticulation was reached at <10 minutes; however, this was shown to be highly dependable on the light source intensity. Oscillatory rheology confirmed that DexGMA and DexGMA/HA formulations act as viscoelastic gels, with a linear viscoelastic region (LVR) extending up to 5% oscillation strain and a yield point at 11%.

DISCUSSION & CONCLUSIONS: Initial results suggest that DexGMA/HA hydrogels will be suitable to progress into testing as a promising bioink with a potential to replace the traditional UV-crosslinkable, methacrylate-based bioinks. Ongoing biocompatibility assays of multiple bioprinted formulations will be followed by histological and RT-qPCR studies, alongside further rheological and mechanical characterisations.

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Keywords: Biofabrication, Cartilage / joint and arthritic conditions

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3D bioprinting of multi-layered aortic-like constructs with a new double-crosslinked decellularized ECM bioink

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INTRODUCTION: Cardiovascular diseases represent the main cause of death worldwide, causing about 17.9 million deaths yearly. Peripheral arterial disease, abdominal aortic aneurysm, and aortic dissection have been the major medical problems affecting human large vessels. Nowadays, the 3D bioprinting technique has been exploited due to incomparable advantages to provide a wide scope for treating vascular diseases. It permits to produce, in a single-step process, living tissues extruding hydrogels with embedded cells, allowing precise, reproducible, and controllable layer-by-layer deposition of different cell types in the custom geometry of the construct. The combination of the advantages of the 3D bioprinting, decellularization process, and different cell species is the strength of this study.

METHODS: Starting from 6-month pigs aortas, a decellularization protocol was optimized to remove cellular and nuclear components from tissues. From the treated samples, a decellularized powder was produced by cryomilling under N₂, and then, solubilized with pepsin digestion. To produce bioinks, several solutions, based on gelatin, alginate, and digested dECM, were studied. After a design of aorta structures performed with SolidWorks, the constructs slicing is done using the software CELLINK HeartWare, which allows generating the G-Code. A doubled-crosslinking process was optimized to obtain a stable structure.

RESULTS: The validation of the decellularization process was performed through DNA quantification, and DAPI, and hematoxylin and eosin staining. It was necessary to optimize the composition of a bioink able to withstand the printing of a segment of tubular construct up to about 20 mm (40 layers) and to reproduce the multicellular complexity. The suspension resulting from 1.2% w/v gelatin, 6% w/v alginate, and 0.66% w/v dECM combined with a pre-printing crosslinking phase with 0.5% CaCO₃ and 1.2% of GDL and a post-printing crosslinking with 1% CaCl₂, was able to produce the desired structures. The dimensions of the printed structures are very close to those established in the design phase.

DISCUSSION & CONCLUSIONS: With this bioink, it is possible to print tubular structures capable of maintaining the desired structure, despite the increase in the number of layers, avoiding the overlap of the double ink in the thickness. The parameters optimization allows the gain of structures characterized by a height of 40 layers corresponding to 20 mm. Comparing the theoretical and the real structures, they are very close to each other. Moreover, by tailoring the printing parameters and the amount of dECM, the desired mechanical properties can be met. The next step includes using endothelial cells and smooth muscle cells.

Keywords: Biomaterials, Polymers - natural / synthetic / responsive

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Sacrificial melt electrowriting creates tailored out-of-plane porosity

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INTRODUCTION: Melt electrowriting (MEW) is an additive manufacturing technique whereby high-resolution fiber scaffolds with tunable geometries have shown to be effective for various tissue applications and in vitro 3D culture models. Since MEW operates on layer-by-layer fabrication by continuous deposition of a polymer fiber, the walls of the constructs do not typically have out-of-plane porosity between the fibers in a reproducible or controllable fashion. This results in limited cellular interconnectivity throughout the construct since cells are unable to assemble and connect through the walls, but rather under or over the walls. We hypothesize that by creating out-of-plane porosity in MEW constructs, that cell connectivity will be enhanced. To achieve this, isomalt, a sacrificial material was co-printed during construct fabrication, producing tailorable pores in the z-plane.

METHODS: Isomalt has previously been investigated for use in MEW[1]. In this study, poly-ε-caprolactone (PCL) and isomalt were co-printed (RegenHU 3DDiscovery). Isomalt fibers were melt electrowritten perpendicularly to the PCL fibers to create out-of-plane pores in the PCL walls. The out-of-plane porosity was controlled through manipulating the diameter, the spacing distance (from 50 μm–200 μm) and the number of stacked isomalt fibers (1-24 stacked fibers high). The isomalt fibers were removed gently by water immersion post-printing. The resulting out-of-plane pores were visualized and measured using scanning electron microscopy (∠90°, Phenom Desktop SEM, ThermoFisher Scientific).

RESULTS: Co-printing of PCL and isomalt was successful and out-of-plane pores were achieved. Control over the size and geometry of these transversal pores was explored and it was determined that pore shape and size variations could be achieved by adjusting the stacking number and spacing of the isomalt. Through controlling the isomalt fiber spacing (using 1x layer), the resulting ‘bridged’ pores achieved a width range of 88–175 μm. Pores controlled by stacking of isomalt fibers resulted in a broader range of sizes. Widths of these pores measured between 34-438 μm and heights (without creating peripheral pores) of 3-245 μm.

DISCUSSION & CONCLUSIONS: This study opens a new axis for customization within MEW constructs, which has potential to improve infiltration of cells within the constructs, as well as enhance the cellular connectivity throughout reinforced tissue constructs. This method could be applied to many tissue engineering approaches using MEW constructs, for example to enhance vascularization by improved interconnectivity.

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Keywords: Fibre technology, Additive manufacturing

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3D Printing Technology in Tendon Tissue Regeneration: Scaffolds for Tissue Reinforcement and Growth Factor Delivery

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INTRODUCTION: The regeneration of damaged tissues is a very complex tissue-dependent process. Its understanding is fundamental for the development of increasingly complex therapies. One of the elements involved in the process are growth factors. They are signalling proteins with a wide variety of biological functions. Its use in clinics has been limited because the infiltration of the factors do not ensure their biological effect on the damaged tissue (they can be distributed to the rest of the body). To solve this limitation, different alternatives have been proposed with a wide variety of results. The objective of this work was to develop a scaffold through 3D printing that gives structural reinforcement in cases of partial tendon injuries and also serves as a controlled delivery system of growth factors that improve tissue regeneration. To this end, two growth factors that play an important role in the early phases of tendon regeneration have been selected: Vascular Endothelial Growth Factor (VEGF) and Platelet-Derived Growth Factor-BB (PDGF-BB).

METHODS: To characterize and determine the *in vitro* effect of the system, the following tests were carried out: (1) determination of the stability of these factors in medium at 37°C by ELISA, (2) incorporation of the factors in the developed ink, (3) obtaining of the scaffolds by 3D printing, (4) determination of the release profiles of the factors from the scaffold by ELISA, (5) determination of the *in vitro* effect of released VEGF in a vascularization assay with Human Umbilical Vein Endothelial Cells and of released PDGF-BB in a cell migration assay with Human Adipose-derived Stem Cells.

RESULTS: The obtained results allowed to establish that both growth factors maintained their biological activity *in vitro* after being incorporated into the ink, printed and released from the scaffold.

DISCUSSION & CONCLUSIONS: To conclude, the structures obtained by 3D printing can also be used as a mechanism for the controlled growth factor delivery in tendon tissue regeneration.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Drug delivery

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Translating resolution: Altering micro-computed tomography outputs for 3D bio-printing

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INTRODUCTION: This study aims to characterise the microarchitecture of mammalian bones to develop a physiologically relevant 3D printed cellular model of trabecular (Tb) and cortical (Cb) bone with suitable resolution. Micro-computed tomography (Micro-CT) imaging allows visualisation and quantification of micro/macro-architecture of *ex-vivo* bone from a variety of species and bone types. The outputs include 3D *in-silico* models that can be rendered in computer assisted design (CAD) software to match resolution capabilities of a 3D extrusion-based bio-printer (BioX, CellInk™). The creation of the biomimic 3D *in-vitro* bone model was achieved by incorporating live cell 3D printing. Due to the organic components of the 3D printed bone bio-ink plus cells, the model was imaged via Micro-CT to assess stability, fidelity, and structural changes due to cellular remodelling.

METHODS: *Ex-vivo* bone samples (tibia, femur, lumbar and caudal vertebrae) from a variety of species (rat, mouse, porcine and bovine) were processed, imaged with Micro-CT with voxel sizes between 9 - 14 µm and reconstructed to determine the morphometry of Tb and Cb. A volume of interest was chosen for inclusion of variation between species. The models were processed *in-silico* to a suitable resolution for bio-printing using CAD assisted software. 3D Models with known dimensions were then printed using Bone Bioink (CellInk™) and osteoblast cells (MC3T3-E1), using various printing parameters to determine optimal printing fidelity to meet intended resolution. *In-vitro* 3D models were cultured and assessed at various time-points using Micro-CT followed by histology.

RESULTS: Multi-species analysis of Tb and Cb highlighted no significant difference in the morphometric outputs for both mouse and rat, including Tb. Thickness (mm) of the tibia and femur. The average Tb. Thickness for mouse and rat combined was 0.067 mm for tibia, and 0.068 mm for femur. The Tb. Thickness of lumbar vertebrae of mice (0.071 mm) was significantly lower than rat (0.104 mm). The variance in Tb thickness influenced the print fidelity, as the smallest diameter of nozzle size (22G, 0.403 mm) was approximately 4 x larger than the thickest Tb of any species measured, limiting resolution capability. The 3D model was therefore rendered in CAD software to allow the highest achievable resolution scaling Tb. Thickness of rat femur by a factor of 10 to achieve a printable Tb thickness width of 0.68 mm matching the achievable resolution of the 3D printer.

DISCUSSION & CONCLUSIONS: The rendered resolution allows viable cells to be printed to create a physiologically relevant dynamic 3D bone model.

Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)

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Co-Culture of Podocytes and Glomerular Endothelial Cells using Melt-Electrowriting – towards an *in vitro* glomerular filtration model

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INTRODUCTION: A functional *in vitro* model of a glomerular filtration unit could facilitate a better understanding of various kidney diseases and offer an insight into the potential nephrotoxicity of new drugs. Using melt-electrowriting (MEW) thin mesh structures, which can be seeded with glomerular cells and are stable enough to resist shear forces present in native kidney, were fabricated. Additionally the complex crosstalk between glomerular cells is heavily dependent on the basement membrane which can be mimicked by decellularized extracellular membrane (dECM).

METHODS: MEW with medical grade polycaprolactone (PCL, Corbion) was performed on a BioScaffolder 3.1 with a Melt Electrowriting head (GeSiM mbH) using a 250 µm needle outlet.

Printing parameters used to prepare meshes with a fibre diameter of 6 µm, layer angle of 30°, and 10 layers were 73°C, 5.3 kV and 22 mm/sec. The hydrophilised meshes were coated with FCS, collagen I from rat (Meidrix Biomedicals GmbH), or glomerular dECM from goat.

Coated meshes were seeded with immortalized podocytes (Evercyte), and with conditionally immortalized glomerular endothelial cells (ciGEnC) (1). Differentiation in podocytes was induced with retinoic acid, in ciGEnC by culture in 37°C.

Cells were analysed for layer density via transepithelial/transendothelial electrical resistance (TEER), specific marker expression via immunohistochemical stainings and qRT-PCR, specific morphology via SEM, proliferation by incorporation of EdU, and cell number by DNA quantification. In co-culture experiments, the cell type seeded last was pre-stained with Dil.

RESULTS: We established a seeding and culture regime which allowed both cell types to form a confluent layer on the coated MEW-meshes, shown by a strong increase in electrical resistance; cell proliferation was also observed via increasing DNA-content over time. Differentiation strongly reduced proliferation in both cell types to <5%, but a small fraction of proliferating nuclei remained in all conditions. Expression of CD31 and fenestrations in ciGEnC increased with differentiation. Podocytes showed the typical foot-processes and expressed the specific markers Nephryn, Podocin, and Synaptopodin, expression did not increase with differentiation though.

DISCUSSION & CONCLUSIONS: This study introduces a culture regime and baseline characteristics of glomerular cells cultured on coated MEW-meshes as groundwork for the establishment of an *in vitro* glomerular filtration model.

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REFERENCES: 1. Satchell et al. 2006, DOI: 10.1038/sj.ki.5000277

Keywords: 3D printing and bioprinting, Kidney

PP-341

3D-printed nanoengineered substrates to advance CAR-T cell production for application in immunotherapy

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INTRODUCTION: Gene delivery is a critical step in cell-based therapies and different approaches are being proposed to assist the intracellular delivery. Currently, viral vectors are the most clinically advanced delivery method due to their high efficiency and specificity. For ex vivo application, lentiviral transduction of T cells is a prominent example that is being used for adoptive immunotherapy of cancer, where tumor recognition function is instructed by induced expression of specific T cell receptors. [1]

Here, we propose a novel biomaterials-based approach to improve transduction efficiency and cell viability. We produced Chimeric Antigen Receptor T cells (CAR-T cells). The novelty of the approach lies within the use of biomechanical cues induced by surface topography of engineered biomaterials. We have recently shown that biomechanical cues can be exploited by T cells to improve their fitness and function. [2] Engineered biomaterials could potentially optimize and modulate T cell behavior and our preliminary data already indicates promising results when comparing against current clinical approaches using microbeads.

METHODS: In an effort to meet scalability, and long-term physical and chemical stability requirements, we deployed a material-based platform suitable for clinical use. Specifically, medical grade porous Polycaprolactone (PCL) was fabricated by direct ink printing with an extrusion bioprinter, using a novel manufacturing approach that enabled production of structures with defined, engineered porosities across a variety of length scales. Induction of CAR-T cells by nanoporous substrates was performed using lentivirus transfection and we assessed the extent of enhanced transduction efficacy for CAR genes (CD19-specific CAR-T cells).

RESULTS: The method introduced in this study allowed 3D printing of porous scaffolds to create designed structures with up to 95% porosity and pore sizes ranging from 100 nm to microscale in a controllable fashion. The printed structures were used for activation and transfection of T cells with CAR constructs and a comparison between nanomaterial-assisted transduction versus conventional approaches for CAR T cell manufacturing was made.

DISCUSSION & CONCLUSIONS: In summary, we show here that 3D printed nanoporous PCL constructs promote the uptake of the lentiviruses by T cells, thereby boosting T cell transduction. Enhanced virus particles uptake by T cells is pore size-dependent and we will discuss possible mechanisms of action.

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Keywords: 3D printing and bioprinting, Biomechanics / biophysical stimuli and mechanotransduction

PP-342

Printing into Matrigel: novel bioprinting techniques to create 3D models of pancreatic cancer

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INTRODUCTION: Bioprinting is a versatile tissue engineering technique which has the potential to create complex three-dimensional (3D) biological models. Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest survival rates of all cancers, partly because these tumours are characterised by a very stiff desmoplastic stroma, which leads to increased drug resistance. Recreating the stiffness and cellular organisation of the tumour microenvironment could lead to the faster development of more effective therapies, as well as the better understanding of the disease processes.

METHODS: In this study, a CELLINK BioX 3D bioprinter was used, exploring methods using extrusion printheads and an inkjet electromagnetic droplet (EMD) toolhead, in order to deposit controlled amounts of bioink. The biomaterial, consisting of 10% methacrylated gelatin (GelMA) with 0.05% LAP was mixed with pancreatic cancer cells (BxPC-3 and CFPAC-1) expressing green fluorescent protein (GFP) or red mCherry. The bioink was dispensed, using 10-80kPa pressures, into either a fluid hydrogel with self-healing properties (gellan gum suspension bath) or into Matrigel, which allowed free movement of the printing nozzle on all three axes, eliminating the requirement of layer-by-layer material deposition. The GelMA based constructs were crosslinked photochemically using a 405nm flood light for 5 minutes, while the Matrigel models were further polymerised at 37°C for one hour. Cell viability and morphology was assessed by 15µm propidium iodide (PI) staining as well as daily microscopy images.

RESULTS: Optimising these techniques yielded reproducible 3D structures with high precision cell deposition and extended cell viability of several weeks. Suspension bioprinting and printing into Matrigel allowed us to create multilineage constructs with defined cell patterning, however, bioprinting in the suspension bath displayed poor reproducibility and precision. One of the main challenges of bioprinting remains resolution, however, we were able to reproducibly place droplets in precise locations into Matrigel, thus creating well defined areas of cells with diameters ranging from 500µm to 1,500µm. Furthermore, preliminary studies of the effects of stiffness on cells, show a decrease in cell movement and viability in a stiff environment (i.e. caging-in).

DISCUSSION & CONCLUSIONS: Our future aims focus on manipulating the construct's stiffness to examine drug penetration and resistance, as well as the effects of hypoxia on the cells at the core. Overall, considering that Matrigel is one of the most widely used hydrogel for 3D cell culture, creating a Matrigel based complex multilineage in vitro 3D model using bioprinting offers countless tissue engineering opportunities, which would better mimic the in vivo microenvironment.

Keywords: 3D printing and bioprinting, Cancer

PP-343

Developing 3D printing models of brain tumours

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INTRODUCTION: Glioblastoma is the most common form of malignant primary brain tumour and accounts for over 40 % of cases. Unfortunately, it is also one of those with the worst prognosis. One of the reasons why glioblastoma continues to have one of the lowest survival rates to this day is due to the lack of more effective therapies that can pass clinical trials. To improve this situation, we need in vitro models capable of accurately predicting tumour microenvironment. To this end, new bioprinting technologies can biofabricate clinically relevant biomimetic tissues which can accelerate drug discovery and additionally serve as a platform for personalized medicine.

METHODS: We generated a series of models in which we bioprinted cells from aggressive brain cancers embedded in bioinks based on different materials, including sodium alginate, fibrin, hyaluronic acid methacrylate (HA-MA) and decellularised extracellular matrix (dECM). We tested whether these materials could mimic the natural microenvironment of those tumours.

RESULTS: Alginate has been used extensively in bioprinting due to its favourable rheological properties acting as a Bingham plastic, meaning that it has a pronounced shear-thinning behaviour. However, the lack of cell adhesion motifs implies the need to functionalize it if it is meant to be used as bioink. In an attempt to functionalize alginate, other authors have combined it with fibrin. Our preliminary experiments show that although fibrin improves the properties of alginate as a bioink, printability requirements limit its biocompatibility. In ongoing experiments we focused on HA-MA as an alternative bioink for our models, given the natural presence of hyaluronic acid in the brain. As an additional advantage, this material can be photo-crosslinked, facilitating its bioprinting. To further optimize it, we aim to improve HA-MA's properties by combining it with porcine brain dECM, increasing the diversity of proteins that are part of the brain microenvironment beyond hyaluronic acid.

DISCUSSION & CONCLUSIONS: The project will test whether these bioprinted models can recreate the brain's microenvironment and provide drug testing data with closer results to human disease than current, simpler alternatives. We hope that a photo-crosslinkable bio-ink based on hyaluronic acid and dECM will offer a better balance in terms of printability and biomimicry. It aims to deliver new technology and methods to study the complex biology of brain cancers and accelerate the provision of new drugs to patients.

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Keywords: 3D printing and bioprinting, Cancer

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3D Bioprinting Tissue Engineered Meniscal Constructs

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INTRODUCTION: Meniscal injuries are the most commonly recorded knee injury by orthopaedic clinicians, affecting over 1.5 million people across Europe and the USA. Injury can greatly reduce knee joint mobility and quality of life, frequently leading to osteoarthritis. Tissue engineered strategies have emerged in response to a lack of viable treatments for meniscal pathologies. These strategies involve the combination of an engineered supporting scaffold with the incorporation of cells and growth factors to stimulate repair and regeneration of damaged meniscal tissue. However, to date, constructs mimicking structural and functional organisation of native tissue whilst promoting deposition of new extracellular matrix remains as a bottleneck in meniscal repair. 3D-bioprinting allows for deposition and patterning of biological materials with high spatial resolution. This project aims to develop biomimetic meniscal substitutes, capable of restoring knee articulation.

METHODS: Human meniscal tissue was characterised to effectively inform the design of biomaterials for bioprinting meniscal constructs with appropriate structural and functional properties. Histology was performed on native tissue to investigate tissue architecture and matrix components. Gene expression analysis was employed to investigate cell populations across meniscal tissue. 3D laser scanning was employed to acquire external geometrical information and a 3D printed meniscus was fabricated using extrusion-based printing. Bioink suitability was investigated through regional meniscal cell encapsulation, in blended alginate/collagen hydrogels, with the incorporation of TGFβ and/or CTGF and assessed through rheology, scanning electron microscopy, histology, immunohistochemistry and gene expression analysis.

RESULTS: Histological analysis revealed regional variations in collagen fibre orientation and matrix composition. Cells from inner and outer regions of tissue revealed distinct gene expression profiles, with loss of phenotype during in vitro expansion. 3D printing was able to produce a scaffold that accurately replicated meniscal geometries. Encapsulating cells from inner and outer regions of the meniscus into hydrogels revealed a recovery in cell phenotype. Incorporating growth factors into the bioink's stimulated cellular re-differentiation and improve zonal functionality.

DISCUSSION & CONCLUSIONS: Meniscus biofabrication highlights the potential to print patient specific, customisable meniscal implants. Future work will involve the regional deposition of cells and growth factors. Achieving zonally distinct variations in cell and matrix depositions will highlight the ability to fabricate a highly complex tissue engineered construct.

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Keywords: 3D printing and bioprinting, Musculoskeletal (inc ligament / tendon / muscle / etc)

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Collagen-based bioinks for tissue engineering and *in vitro* models

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INTRODUCTION: 3-D bioprinting is one of the most promising bio-fabrication technologies as it allows to design complex 3-D structures mimicking living systems with high resolution and reproducibility¹. Natural hydrogel systems have been employed as bioinks thanks to their inherent biocompatibility and the ability to replicate the extracellular environment. In this context, collagen and collagen-derived materials (e.g. gelatin) have played a central role as collagen is the most abundant protein of several human tissues (skin, bone, lung). Otherwise, Gelatin-Methacryloyl (GelMA), a photo-crosslinkable form of gelatin, has recently been investigated as bioink due to its rapid crosslinking rate and excellent thermostability². Here we describe the development and characterization of collagen-based hydrogels that allow to bioprint biomimetic 3-D structures with high cytocompatibility and modulable mechanical properties.

METHODS: A thermo-sensitive hydrogel was obtained by dispersing type I collagen powders in an acidic culture medium. The sol-gel transition was induced by neutralizing the pH and rising temperature to 37°C. Alternatively, the formulation of a photo-crosslinkable hydrogel was optimized by adjusting the GelMA and photo-initiator ratio and the duration of light exposure. Rheological characterization was performed to investigate the gelation properties and mechanical stability of the different hydrogels. Preliminary printing tests were conducted to evaluate the printability of developed formulations and to optimize printing parameters. Finally, fibroblasts were encapsulated into the hydrogels and cell viability was assessed by Cell Titer blue viability assay up to 14 days.

RESULTS: The rheological measurements demonstrated the sol-gel transition of collagen and GelMA pre-hydrogel solution due to temperature and light irradiation, respectively. The increment of storage modulus (G') values to reach a stable plateau identified the elastic response of the materials. The printing tests were conducted by tuning printing parameters. Well-defined porous structures were obtained after printing. For all the tested conditions, the metabolic activity of cells increased over the incubation time, indicating an active proliferation within the hydrogels.

DISCUSSION & CONCLUSIONS: The reported results suggest that the realized structures can be applied as extracellular matrix (ECM)-mimic substrate for tissue engineering and *in vitro* model applications.

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Keywords: Biofabrication, In vitro microenvironments

PP-346

4D bioprinting morphing tissue to sculpt cell and ECM alignment

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INTRODUCTION: During embryonic development, organs emerge through highly dynamic processes driven by shape transformations that sculpt their final shape and function (Xiong, Fengzhu, et al., 2014). Despite this, existing approaches to bioprinting typically employ static hydrogels that are not capable of supporting morphogenetic shape changes (Mirdamadi, Eman, et al., 2019). To overcome this limitation, the objective of this work was to develop an extracellular matrix (ECM)-based bioink that undergoes cell-mediated shape-morphing. We hypothesized that the bioink could be used to create tissues that undergo programmable morphing based on spatial variation in active cell forces. Further, we hypothesized that tissue-level shape-morphing could be used to sculpt internal cell/ECM alignment.

METHODS: First, we combined collagen type-I (Advanced Biomatrix™) and hyaluronic acid (Contipro™) to create a fibrous ECM bioink with shear-thinning properties. Bioprinting in granular support hydrogels was used to create various geometries such as beams, sheets, and tubes, fundamental structures ubiquitous in early organ development. The cell (2-10 million/ml), ECM (2.4-8 mg/ml collagen), and support hydrogel (60-100% packing density) concentrations were varied to explore their influence on tissue shape-morphing. Confocal microscopy was used to visualize and quantitatively map shape-morphing patterns at the cell, ECM and tissue scales over fourteen days of culture. To explore the mechanism of shape-morphing, the bioprinted tissues were treated with a range of inhibitors that disrupt actin cytoskeleton and myosin-II activation (e.g., Y-27632, blebbistatin and cytochalasin D). Statistical comparisons between groups were performed using two-way ANOVAs.

RESULTS: Bioprinted tissue beams, sheets, and tubes underwent significant shape-morphogenesis within the support hydrogel over fourteen days. Importantly, the extent of shape-morphing behaviour was tunable, with increased morphogenesis observed at low collagen concentrations (2.4-8mg/ml; 27-9% volumetric reduction) and high cell densities (2-10 million/ml; 27-40% volumetric reduction). Interestingly, the shape-morphing patterns also depended on the initial tissue geometry, with isotropic shrinkage observed in bioprinted tubes and anisotropic bending observed in beams/sheets. Tissue-scale shape-morphing also influenced internal cell-ECM organization, with alignment emerging along the longitudinal axis of bending beams/sheets and circumferential alignment emerging in shrinking tubes. Finally, high-resolution confocal and SEM microscopy, along with Rho/Rock-pathway inhibitors, demonstrated that tissue shape-morphing was driven by active cell forces resulting from cytoskeletal actomyosin contractility.

DISCUSSION & CONCLUSIONS: An ECM-based bioink has been developed for bioprinting tissues that undergo programmable actuation based on spatial variations in cell-generated forces. These tunable shape-morphing behaviours gave rise to regional variations in cell and ECM alignment, which provides a new tool to sculpt the development and maturation of bioprinted tissues.

Keywords: 3D printing and bioprinting, Biofabrication

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Generation of high cell density bioinks for bioprinting of functional cartilage grafts

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INTRODUCTION: Tissue engineering (TE) strategies aim to regenerate tissues which have lost significant functionality due to physical damage or disease. Current approaches fail to recapitulate the highly organised structure and extracellular matrix (ECM) composition of complex tissues such as articular cartilage, motivating the exploration of novel TE strategies. 3D Bioprinting enables the precise deposition of bioinks in a spatially organised, three-dimensional manner, potentially enabling the engineering of complex tissues and organs. The objective of this study is twofold. The first objective is to explore the impact of bioink cell seeding density on the engineering of articular cartilage grafts. The second objective is to explore the potential of the freeform embedded suspended hydrogel (FRESH) technique¹ for the bioprinting of low viscosity bioinks containing high concentrations of bone-marrow derived mesenchymal stem/stromal cells (MSCs).

METHODS: MSCs were encapsulated in a rapidly degrading oxidised or non-oxidised alginate based bioinks at specific cellular densities (2, 4 or 6×10^7 cells/ml). These cell laden bioinks were deposited into agarose moulds and cultured in chondrogenic medium for 6 weeks. Constructs were then assessed biochemically and histologically. A degradation study was undertaken to assess how differently oxidised alginate inks degrade over time. For the embedded printing process, material characterisation and optimisation of various bioink compositions and an adapted FRESH support bath matrix were undertaken.

RESULTS: Both the medium (4×10^7 cells/ml) and high (6×10^7 cells/ml) cell density bioinks supported superior chondrogenesis to the low density bioink (2×10^7 cells/ml), with statistically higher levels DNA, sulphated-glycosaminoglycans (sGAGs), and collagen production. Histological evaluation also confirmed the medium and high-cell density inks supported robust chondrogenesis. No differences in cellular viability were observed. The 4% oxidised alginate inks degraded completely over a 4-week period.

Following optimization, it was possible to generate a FRESH support bath with an average, uniform microparticle size of $20 \mu\text{M}$. It was possible to accurately deposit alginate based bioinks into such a support bath, with relatively high resolution ($<100 \mu\text{M}$ filament diameter) and fidelity in single material, acellular printing trials.

DISCUSSION & CONCLUSIONS: Initial work has been undertaken to begin the development of a novel bioprinting platform, including material, bioink, print process optimisation and characterisation. Future studies will explore multi-material, 3D embedded bioprinting of physiologically representative constructs with spatial-temporal defined alginate boundaries, with a view to engineering cartilaginous tissues with user-defined collagen network organisation.

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Keywords: 3D printing and bioprinting, Biomaterials

PP-348

Electroconductive PCL-PEDOT:PSS 3D-extruded scaffolds for electrical stimulation of bone cells

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INTRODUCTION: Bone tissue engineering remains a core area of research in regenerative medicine. Bone tissue is a terminally differentiated tissue rich in collagen and hydroxyapatite, and its regenerative potential is limited. Bioprinting allows the production of 3D porous scaffolds capable of osteointegration and regeneration. The application of electrical stimulation (ES) using electroconductive materials can further improve osteoregeneration. The goal of this work was to develop an optimal coating for extruded poly(ϵ -caprolactone) (PCL) scaffolds suitable for the culture and electrical stimulation of bone cells.

METHODS: PCL films and scaffolds (300 μ m for filament diameter and pore size) were extruded using an Original Prusa i3 MK3 (0.25 mm nozzle) and partly hydrolyzed in NaOH solution. Different solutions containing poly(ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS), cross-linkers (3-glycidyloxypropyl)trimethoxysilane (GOPS) and divinyl sulfone (DVS), and gelatin were tested for dip-coating. Performance was evaluated through electroconductivity and respective long-term stability, mechanical properties, and in vitro mineralization. Mesenchymal stem/stromal cells (MSCs) were differentiated into osteoblasts with electrical stimulation for 21 days. Differentiation was evaluated through calcium deposition, immunofluorescence, and gene expression.

RESULTS: Coating of PCL with PEDOT was only possible when cross-linkers were used. All combinations (GOPS, DVS) without and with gelatin were electroconductive (11 S cm⁻¹ for PCL(NaOH)-PEDOT:DVS-Gel) and stable in PBS for up to 21 days. Young's module of scaffold PCL(NaOH)-PEDOT:DVS-Gel was 11 MPa. Overall, the PEDOT:PSS coating greatly improved in vitro mineralization of the scaffolds. ES of cultured cells on PCL(NaOH)-PEDOT:DVS-Gel improved gene expression of Osterix, Collagen I and CACNA1C, indicating improved bone cell maturation and suggesting improvements in extracellular matrix production. Finally, calcium deposition by the cells on the scaffold was greatly enhanced.

DISCUSSION & CONCLUSIONS: In this work we developed PEDOT:PSS-coated 3D-extruded PCL scaffolds for bone tissue engineering applications. Application of ES greatly enhanced MSC differentiation and improved calcium deposition. Our results show the potential of such strategy for future clinical applications.

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Keywords: Biomaterials, Electroactive materials

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Development of a platform to generate thousands of 3D spheroids for bioprinted liver model for drug efficacy and safety assessment

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INTRODUCTION: Drug discovery is still a risky, expensive, and time-consuming process. Bioprinted liver models are promising tools to produce biomimetic constructs and efficiently assess the efficacy and safety of new drugs. However, for these models, a large number of 3D spheroids is required which is not trivial to achieve.

Currently, microwell array plates (either commercially available or hydrogel-based custom designs) are used, with limitations in terms of number of spheroids produced, lack of homogeneity, spheroid size and shape and cost [1]. The development of a reusable platform to produce thousands of spheroids, with enhanced control of spheroid size and shape can circumvent all these limitations while allowing bioprinting of liver constructs for drug screening.

METHODS: An acrylic mould with microcavities of 250 µm was developed by means of subtractive micromilling, with dimension fitting a well of a 6-well plate. A negative replica of the mould was manufactured with polydimethylsiloxane (PDMS), and the same material used to produce the positive microcavity insert, with a double casting method enabled by plasma treatment and alcohol passivation [2]. HepG2 cells were seeded to obtain spheroids.

The Aspect Biosystems bioprinter was chosen to produce an alginate filament containing spheroids, crosslinked with calcium chloride (125 mM). A LIVE/DEAD assay was performed to assess spheroid viability inside the filament after 3 days.

RESULTS: Both negative and positive PDMS stamps were successfully developed, presenting the desired microcavities reproducing the original acrylic mould. The replica was used to seed HepG2 cells and after less than 48h, compact cell spheroids were observed. Thousands (up to 15000) of spheroids were easily retrieved from each well, and the ones with a diameter below 100 µm were bioprinted in alginate filaments. The filaments remained stable and the spheroids viable.

DISCUSSION & CONCLUSIONS: We were able to design a platform to generate thousands of spheroids, in a simple and fast manner. The cell aggregates form easily compact spheroids over time that can be retrieved without digestion or trypsinization. The spheroids can be bioprinted inside alginate filaments and remain viable for days, which is promising to develop bioprinted liver models for drug screening.

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Keywords: Biofabrication,

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Combining a tissue-specific hydrogel with polysaccharides to formulate bioinks for 3D bioprinting

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INTRODUCTION: Polysaccharides are extensively used in bioink formulations for extrusion-based 3D bioprinting due to their biocompatibility and processability. However, their low bioactivity is a major drawback. Tissue-specific decellularized extracellular matrices (dECMs), in turn, contain biochemical components that provide the ideal microenvironment for cellular attachment, proliferation, migration and differentiation. Herein, we combined the viscoelastic behavior of xanthan gum (Xan) and the fast crosslinking ability of alginate (Alg) with the unique bioactivity of dECM to achieve a bioink with good printability and superior biological performance.

METHODS: Alg and Xan were combined at different mass proportions - 2:1 (R1); 1:1 (R2) or 1:2 (R3) - and mixed with a lung dECM hydrogel (MatriXpec™ Lung, TissueLabs, Switzerland). The gels were combined with concentrated culture medium (DMEM 10x) (gel:medium = 9:1, v/v) to obtain the final bioink formulation and secondary crosslinking was performed with CaCl₂ 50 mM. As control, a formulation containing phosphate buffered saline instead of dECM was prepared. Rheological properties were analyzed using a rheometer. Bioink viscosity was determined at 25 °C with shear rate varying from 40 to 1000 s⁻¹. Yield stress measurements were performed by oscillatory tests, with stress ranging from 1 to 300 Pa. Post-printing recovery was evaluated by applying a shear stress of 1 s⁻¹ for 25 s; 700 s⁻¹ for 50 s and monitoring the viscosity during 300 s. Printability was assessed by filament fusion and collapse tests using a 3D bioprinter (TissueStart™, TissueLabs, Switzerland). Cytotoxicity was assessed with FMM-1 fibroblasts by resazurin assay.

RESULTS: All formulations present a pseudoplastic behavior (viscosity decreases as shear rate increases) and R1 is the most viscous bioink. The ultimate yield stress was higher for R1 (103.3 Pa) and similar between R2 and R3 (~40 Pa). Post-printing viscosity recovery reached 96% for R1 and less than 87% for R2 and R3. Printability of R1 was satisfactory, with minimum material spreading and no collapse of printed filaments between pillars of 6 mm distance. This formulation did not show any toxicity to fibroblasts. Resazurin reduction, indicative of cellular metabolism, was similar to the negative control (cells grown with culture medium).

DISCUSSION & CONCLUSIONS: Bioinks with a range of rheological properties and good printability were obtained from the combination of polysaccharides and dECM.

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Keywords: Biomaterials, Biofabrication

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Extrusion-based 3D printing of spongiosa-like osteoinductive scaffolds based on calcium phosphate and PCL

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INTRODUCTION: In an aging society, where degenerative diseases, such as osteoporosis and osteoarthritis are becoming more common, individualized bone grafts are becoming increasingly important. Bone defects, often have irregular shapes resulting in the need for new technologies to produce individual implants. Extrusion-based 3D printing is an additive manufacturing method to create complex and individualized bone constructs which can be seeded with autologous cells from the patient.

METHODS: In this study, we established an extrusion-based printing process to produce osteoinductive scaffolds based on the biocompatible thermoplastic polycaprolactone (PCL) combined with calcium phosphate (CaP) as an osteoinductive component.

The internal structure of the scaffolds resembles a bone-like spongiosa with irregular interconnected pores (diameter: 2 mm +/- 0.2 mm), whereas the outer shape is a ring with a diameter of 20 mm and a height of 10 mm. To produce the printing material, the PCL was combined with CaP nano powder (> 150 nm particle size) under heating and printed with the BIO X6 (CELLINK) into a complex 3D structure. After printing, 5 x 10⁶ MG63 (osteosarcoma cell line) or human mesenchymal stem cells were seeded onto the constructs using rotating incubator over a period of 24 h. The cell response was analyzed using different methods. Further, the mechanical properties of the scaffolds were determined.

RESULTS: The MG63 cells attach to the PCL and the PCL/CaP composite scaffolds and proliferate over the investigated time frame (14 days), as indicated by DNA quantification. In both, confocal laser scanning as well as scanning electron microscopy, an elongated morphology of MG63 cells was visible. Alizarin red (AR) staining was used to approve the calcification processes of the cells, as well as the integration of CaP particles into the scaffolds. AR indicated freely accessible CaP particles at the material surface, as well as distinct centers of cellular calcification. The composites with CaP influenced the gene expression of the osteogenic differentiation markers indicating significant increase in osteogenic markers collagen type 1 and osteocalcin for the CaP composite material. Further the printed scaffolds revealed bone-like mechanical stability in compression strength tests (PCL: 3.4 +/- 1.3 MPa, PCL CaP: 3.05 +/- 0.99 MPa).

DISCUSSION & CONCLUSIONS: In conclusion, the study shows promising properties of the CaP containing 3D printed bone grafts in terms of mechanical properties, biocompatibility and osteoinductive properties in vitro.

Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)

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3D Bioprinting as an Approach for Homogeneous Cell-Seeding on Natural Scaffolds

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INTRODUCTION: 3D bioprinting is a promising tool for fabricating congenital heart disease relevant constructs that have the potential to grow, remodel and regenerate in vivo. It, also offers the potential to be used as a cell-seeding technique on natural scaffolds, due to its ability to control the positioning and distribution of cells. In this work, we investigated bioprinting as a cell-seeding approach.

METHODS: Various geometries of bioprinted constructs composed of pig thymus mesenchymal stem cells encapsulated by alginate hydrogel were bioprinted on in-house decellularized extracellular matrix amniotic membrane (dECM AM) and commercial decellularized extracellular matrix ProxiCor (dECM ProxiCor) scaffolds. A comparison between their cell viabilities/total number of cells was carried out.

RESULTS: The bioprinting technique succeeded in achieving homogenous distribution of cells in bioprinted constructs as opposed to manual seeding technique. Moreover, no-grid based bioprinted constructs yielded significantly higher cell viability and number of live cells compared to grid bioprinted constructs. There was also a significantly higher number of live cells in dECM ProxiCor compared with dECM AM.

DISCUSSION & CONCLUSIONS: Our results show that 3D bioprinting can accurately position and homogeneously distribute cells on natural scaffolds. This approach holds promise to produce good quality stem-cell patches that could, potentially, be used in cardiac regenerative medicine applications.

Keywords: 3D printing and bioprinting, Stem cells - general

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Fabrication and application of 3D printed Gelatin/oxidized alginate-based cryogels scaffolds for bone tissue engineering

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INTRODUCTION: Natural biopolymers are recognized and widely used for the preparation of cell-loaded scaffolds for bone tissue development due to their excellent biocompatibility and adaptable biodegradation rate. The porosity and average pore size of scaffolds have a significant impact on biological activities such as cell proliferation kinetics and tissue regeneration processes related to extracellular matrix synthesis [1-2]. Here, we present a composite formulation of gelatin and oxidized sodium alginate (Gel/OxAlg) that enables the combination of 3D printing and cryogelation techniques for the straightforward and cost-effective fabrication of biocompatible scaffolds characterized by high porosity and particularly large pores.

METHODS: Scaffolds in different concentrations of gel and OxAlg were prepared using a 3D printer (model: Bio-X CELLINK, Sweden) and processed by cryogelation at -20°C for 24 hours. The printability of the bio-inks, mechanical properties, and porosity of the finished constructs were investigated. Approximately 0.5 million NIH/3T3 (ATCC® CRL -1658™) fibroblasts were seeded onto each scaffold. Cell viability and scaffold morphology were studied during the 21-day incubation period.

RESULTS: Oxidized sodium alginate (OxAlg) was mixed with gelatin (gel) in a 1:1 ratio to prepare four aqueous gel/OxAlg (w/v) bioink formulations with different concentrations: 2.50, 2.67, 2.86, and 3.08 percentages. The resulting scaffolds were immediately cryogelated at -20 °C for 24 hours. After thawing at room temperature, chemically cross-linked macroporous hydrogel scaffolds were prepared. The composition of 2.86% (w/v) (1:1) gel/OxAlg cryogels showed a pronounced mechanically strong gel behavior, a slightly lower degree of degradation, characterized by high porosity and large pore structure ($d > 100 \mu\text{m}$) compared to other compositions. Live/dead staining images confirmed cell viability and SEM images after 21 days on 2.86% (w/v) (1:1) (gel/OxAlg) cryogels scaffolds. Cell viability was reported to be ~91.4%.

DISCUSSION & CONCLUSIONS: These results suggest that low-concentration gel/OxAlg mixtures can be used as inks for 3D-printed hydrogel scaffolds combining the cryogelation method. The distinct 2.86% (w/v) (1:1) gel/OxAlg composition has the highest potential for scaffold fabrication by 3D printing due to its biocompatibility, enhanced rheological properties, and appropriate degradation rate. The cryogels scaffolds shown are characterized by an exceptionally broad porosity structure and long-lasting mechanical stability, which is advantageous for bone tissue engineering applications.

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Keywords: Additive manufacturing, Advanced therapy medicinal products

PP-354

Deployable 3D bioprinting for modeling pancreatic tumour microenvironment with cancer associated fibroblasts

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INTRODUCTION: Three-dimensional (3D) bioprinting as a promising tool enabled rapid and reproducible construction of in vitro cancer models with tumor microenvironments (TME). However, innovations still needed to integrate affordability, user-friendliness, and biological relevance in bioprinting workflow to widen the translational impacts.

METHODS: Here we developed 'Bioarm', a simple, yet highly effective extrusion bioprinting platform, which can be folded into portable form, and rapidly deployed between facilities. Bioarm enabled direct TME reconstruction by printing 3D core-shell tumoroids with cancer cells deposited as a core inside the cancer-associated fibroblasts (CAFs) shell. Being easy-to-manipulate, those 3D printed tumoroids can later be integrated with various simulated TME.

RESULTS: Compared to traditional 2D co-culture models, 3D-printed tumoroids raised a heterogenous population of CAFs and produced de novo synthesized extracellular matrices, demonstrating more in vivo-like characteristics. Embedding the 3D printed tumoroids in an immune cell laden matrix allowed live tracking of the cancer-immune interactions, and subsequent immune perturbation under immunotherapy treatments.

DISCUSSION & CONCLUSIONS: Overall, our deployable extrusion bioprinting workflow could significantly improve the accessibility of 3D bioprinting for replicating TME with complex multi-compartmental architectures and can be potentially used for personalized cancer drug screening platform.

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Keywords: 3D printing and bioprinting, Cancer

PP-355

Combining 3D hydrogel structures with high precision bioprinting

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INTRODUCTION: In recent years, multi-layered hydrogel constructs loaded with biological cells have been successfully utilized for developing biomedical applications. With this approach, hydrogels offer a suitable physical and chemical environment for cells, however accurate cell positioning remains largely unresolved. Here, we combine 3D hydrogel structures with high-precision bioprinted tissue models, where cells are printed on top of hydrogel layers using an open-volume microfluidics technology. As the demonstration of this approach, we produced bioprinted islet microtissues sandwiched between two layers of hydrogel, as well as a basic multi-layered skin tissue model.

METHODS: Bioprinted tissues were produced using microfluidics-based technology as described previously. (1) Thin hydrogel layers (up to 0.5 mm) were formed using gelatin methacrylate (GelMA) or alginate.

RESULTS: To prepare islet microtissues, circular patterns of mouse pancreatic cells (a mixture containing 80% of Min6 and 20% MS1 cells) were bioprinted on a top layer of hydrogel (alginate). These patterns have a typical diameter of approximately 200 μm , mimicking the size of native pancreatic islets. Two hours following the cell bioprinting, the cells were covered with an additional layer of hydrogel, providing suitable conditions for islet development. These constructs were cultured for 7 days, after which a glucose-stimulated insulin secretion assay and live/dead staining were performed to demonstrate the functionality and viability of bioprinted islet microtissues.

As a further demonstration, a multi-layered basic skin tissue model was assembled from three distinct layers of hydrogel (GelMA), whereby cells (HaCaT) were printed on top of the first and second layers. The cell patterns were preserved for at least 24 hours, and the whole multi-layered construct could easily be released from the production frame and transferred for further use.

DISCUSSION & CONCLUSIONS: With the proposed approach we were able to demonstrate a combination of conventional hydrogel structures with high-resolution direct cell bioprinting. This combination of patterning techniques enables for mimicking the complexity of native tissues and advancing current in vitro systems for disease modelling and drug screening, as well as developing novel applications within the area of regenerative medicine.

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Keywords: In vitro microenvironments, Biomaterials

PP-356

Silk-based microfiber structures with well-organized geometries and improved elastic properties

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INTRODUCTION: The link between silk fibroin (SF) chemical composition and material structure is a subject of great interest for (bio)material scientists.¹ The hierarchical secondary structure of SF is responsible for its exemplary mechanical properties. At the nanoscale it combines beta-sheet nanocrystals embedded in a softer semi-amorphous phase, that are assembled into fibrils at microscale and into fibers at millimeter scale. However, with existent silk processing methods, this complex hierarchical structure is destroyed compromising its final mechanical properties.² In this work, we hypothesized that electrohydrodynamic writing of SF-based aqueous solutions and their subsequent post-processing in aqueous salt solutions will improve hierarchical assembly of the SF and allow fabrication of controllable solid SF microfibers with improved mechanical properties in terms of strength and elasticity.

METHODS: SF was extracted from Bombyx mori cocoons and processed with an in-house built electrowriting printing set-up. Key printing parameters (i.e. speed, voltage and pressure) were investigated to obtain square shape scaffolds with straight fibres. Post-printing fibers were treated with a water solution of NaH₂PO₄ (2M) to induce physical crosslinking, determined by Raman spectroscopy. Post-printing accuracy was evaluated analyzing the diameter and the stacking of the fibers with SEM and mechanical properties were analyzed with uniaxial tensile test. To confirm, the biological potential of SF scaffolds, epithelial kidney cells (ciPTEC) were seeded and cultured in vitro on post treated scaffolds.

RESULTS: We demonstrated a solvent-free electrowriting process to obtain SF microstructures. By varying the concentration of SF (15-20% w/v) and the concentration of polyethylene oxide (2.5-3% w/v) (Mw: 600-1000 kDa), straight fibers in squared shaped laydown patterns with a fibre diameter between 5 and 20 µm and a minimum interfibre distance of 400 µm were successfully produced. We observed that by soaking printed scaffolds in a NaH₂PO₄ part of the beta-sheets and alfa-helices showing a stiffness of 250mN and an elastic response at 20% deformation for 10 cycles. Finally, we confirmed that materials used were non-cytotoxic and that ciPTEC cells adhered to fiber scaffolds when coated with L-DOPA and were able to create uniform monolayer characterized by the production of collagen V, typical of healthy epithelial tissue.

DISCUSSION & CONCLUSIONS: In summary, we demonstrated that we can achieve the hierarchical structure of SF from the secondary structure to the fiber organization through an electrohydrodynamic writing and a post-printing process, improving the mechanical properties in terms of strength and elasticity.

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Keywords: 3D printing and bioprinting, Biomaterials

PP-357

Extrusion-based 3D printing of collagen scaffolds with native-like organization

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INTRODUCTION: In vivo, collagen molecules (Col) assemble into an extracellular matrix (ECM) of intertwined fibers.[1] The network organization (density, dimensions, alignment and porosity) is tissue-specific and cannot be fully mimicked in vitro: most work was done in diluted conditions, neglecting the physiological conditions, in particular, the macromolecular crowded environment and confinement forces that affect Col assembly in vivo.[2]

We aim to mimic the ECM organization combining a bottom-up and a top-down approach. We will explore the effect of macromolecular crowders (MMC) on the density and dimensions of Col fibers (bottom-up) and use extrusion-based 3D printing guide the alignment of Col fibers and tune the organization and dimensions.

METHODS: Col constructs were fabricated by extruding different Col-based inks into a gelatin support bath and incubated at 37°C.[3] The effect of the following parameters on the construct resolution and organization was studied by optical, fluorescence and scanning electron microscopies:

- 1) Col ink compositions: acidic solution of Col molecules (sColl) and acidic dispersion of Col native-like fibers (dColl), with and without the addition of different concentrations of polyethylene glycol (PEG), a common synthetic MMC.
- 2) Printing conditions: systematic variation of key printing parameters, i.e. extrusion rate, printing speed and nozzle diameter to study printing resolution.

RESULTS: Col constructs were obtained by extrusion of different ink formulations (sColl, sColl+MMC, dColl and dColl+MMC) in a gelatin support bath. The printed sColl filaments are 2-3 times thicker and less defined compared to dColl. The addition of PEG to the dColl ink leads to increased stability and higher resolution of the printed filaments under the same printing conditions: $(183 \pm 3) \mu\text{m}$ for dColl and $(138 \pm 2) \mu\text{m}$ for dColl+MMC. SEM characterization shows that Col fibers of the dColl+MMC constructs are aligned, while for sColl and dColl, they present an isotropic organization.

DISCUSSION & CONCLUSIONS: Here we show that by optimizing printing parameters and Col-based ink compositions, the macro- and microstructural organization of Col fibers can be tuned. In particular, the addition of MMC improve the printing resolution and, in combination with the printing process, directs the alignment of the Col fibers. Being able to mimic in vitro the organization of collagen in vivo will lead to improved ECM models of diverse tissues, and potentially to implants.

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Keywords: 3D printing and bioprinting, Biomaterials

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A novel eggwhite powder-enhanced bioink stimulates cell proliferation and response in 3D bioprinted tissue substitutes

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INTRODUCTION: Three-dimensional bioprinting has attracted great interest for fabrication of hierarchically structured, functional tissue substitutes with a spatially defined cell distribution. Although considerable progress has been witnessed, certain limitations remain; particularly a lack of suitable bioinks with favourable cell response and high shape fidelity at the same time [1]. Protein-based biomaterials support cellular responses, but are often limited by sources, complex extraction and purification procedures, and high cost. Chicken eggwhite (EW) is a cheap and easily available protein-based material, exhibiting excellent biological properties and a great potential as an alternative biomaterial for tissue-engineering applications [2-4]. Previous study found that the ability of EW to support positive cell response is concentration-dependent [5]. Thus, exploration of bioinks with higher EW content is promising and therefore, egg white powder (EWP) was applied for development of bioinks with the aim of solving these limitations mentioned above.

METHODS: EWP (10%, w/v) was dissolved in PBS as precursor solution; alginate (Alg, 3 %, w/v) and methylcellulose (MC, 9 %, w/v) were dissolved into the precursor solution to obtain AlgMC+EWP ink. Before bioprinting, mammalian cells were suspended in the ink to print cell-laden constructs. Response and biofunctionality of different cell types (e.g. human mesenchymal stem cell line, primary pre-osteoblasts (hOB), osteocytes and fibroblasts) in the scaffolds were explored and evaluated.

RESULTS: The novel EWP-enhanced bioink allowed the fabrication of stable 3D volumetric cell-laden constructs. Viability, adhesion and proliferation of encapsulated cells in the structures were significantly enhanced by addition of EWP; osteogenic gene expression of hOB was partially improved. In addition, the bioink was successfully co-printed together with inorganic calcium phosphate cement ink to fabricate biphasic cell-laden mineralized constructs.

DISCUSSION & CONCLUSIONS: EWP was successfully utilised for preparation of bioinks. The developed bioink exhibited not only good printability and shape fidelity, but also stimulates the response of different cell types. High-strength cell-laden volumetric structures were fabricated by combined printing with an inorganic ink, demonstrating the high flexibility of the developed bioink that could be applied to construct soft and hard tissue substitutes. On the other hand, the developed bioink is cheap, ready-to-use and easily available, offering great potential for biofabrication of tissue substitutes or in vitro tissue/organ models for drug testing.

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Keywords: Biomaterials, 3D printing and bioprinting

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Biocompatibility of 3D bioprinted tissue model prototypes containing sustainably grown coral skeletal particles for orthopedic applications

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INTRODUCTION: 3D bioprinting allows for the creation of reproducible 3D tissue models, tailored for each tissue type through the combination of materials, growth factors, drugs and cells. These models can be applied to tissue engineering, drug discovery and development and disease research. In orthopedics, 3D bioprinted scaffolds offer a wealth of clinical opportunity transitioning from traditional bone void fillers to patient-specific printed implants. Coral skeleton has a long history of use in bone grafting as a biocompatible, osteoconductive material. Challenges around sustainable sourcing, reproducibility and strength have limited its success to date. In this work, we present a bioink containing sustainably grown coral, which can be used to bioprint bone tissue 3D models with osteoconductive functionality.

METHODS: Several coral-bioink formulations were evaluated in this study, including two CellInk bioink bases (nanocellulose-alginate base and gelatin-methacrylate-xanthan gum base) combined with 7-23% (w/w) coral granules (Zoan Biomed) and mesenchymal stromal cells (MSCs). The biocompatibility of the scaffolds with the MSCs was evaluated through live/dead staining and fluorescent microscopy and by measuring the release of lactose dehydrogenase (LDH) into the culture medium. Several formulations of coral-bioinks containing MSCs are being characterized through scanning electron micrograph methodologies including variable pressure imaging using a backscattered electron approach and secondary electron imaging. Properties including porosity, coral distribution and cell-coral engagement are under investigation.

RESULTS: All coral-bioink formulations were highly biocompatible. The MSCs were evenly distributed throughout the bioprinted construct, remaining viable for >8 days after bioprinting as evidenced by abundant green fluorescence and minimal red fluorescence. These findings indicated that the shear thinning behavior of the biopolymers, even with high concentrations of coral, prevented an increased shear stress on cells during extrusion.

DISCUSSION & CONCLUSIONS: Novel coral-containing bioinks have the potential to contribute to strong, highly biocompatible constructs for bone tissue models and regenerative medicine for future orthopedic medical devices. A sustainable, highly reproducible source of coral biomaterial with high mechanical strength provides a viable alternative to synthetic calcium salts in 3D bioprinted applications.

Keywords: Composite materials, Polymers - natural / synthetic / responsive

PP-360

Bioprinting of double network hydrogel for skeletal muscle regeneration

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INTRODUCTION: Sodium alginate (Alg) and gelatin (Gel) are natural polymers, usually obtained from seaweeds and denaturation of collagen via hydrolysis process, respectively. Gelatin has been greatly used as biomaterials in different form such as microparticles, scaffolds, electrospun nanofibers, hydrogel, and three dimensional (3D) bioprinting for drug delivery, wound healing, and tissue regeneration applications¹. Here, we have fabricated the Alg-Gel-based ionically crosslinked double network (DN) hydrogels with varying concentrations of gelatin and bioprinted with mouse myoblast cell line C2C12. Three different compositions of Alg-Gel (Alg:Gel = 1:0, 1:0.25, and 1:0.50 w/w) were fabricated.

METHODS: In this work, we have used bioprinting technique to construct 3D structure. The double network hydrogel with C2C12 cells were 3D bioprinted and cultured for cell viability, metabolic activity, and proliferation. Cell viability, metabolic activity, and cell proliferation were evaluation by Live/Dead (calcein-AM/ethidium homodimer-1), AlamarBlue, and dapi/phalloidin staining.

RESULTS: The results of DN hydrogel exhibited higher compressive strength (17 kPa to 56 kPa), and slower stress relaxation (~20 s to 62 s). The Cryo-scanning electron microscope (CryoSEM) images of Alg, and Alg-Gel hydrogels revealed uniform networks with varying pore size. Furthermore, bioprinted hydrogels samples exhibited high cell viability (~80% to 90%), high metabolic activity, and DAPI/Phalloidin staining also showed significantly higher cell proliferation. The in vitro studies using C2C12 could demonstrate the myogenic differentiation and promote the myotube formation.

DISCUSSION & CONCLUSIONS: These findings highlight that the Alg-Gel-based 3D bioprinted hydrogel exhibits potential as a living therapeutic biomaterial; it is highly effective in supporting live cell proliferation and can promote the myogenic differentiation of C2C12 cells, which will eventually orchestrate to accelerate the skeletal muscle regeneration process.

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REFERENCES: Armstrong, J. P. K., et al., Advanced Healthcare Materials (2016) 5 (14), 1724

Keywords: 3D printing and bioprinting, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-361

Freestanding Magnetic Cell Sheets for Tissue Engineering Applications

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INTRODUCTION: A main challenge in tissue engineering (TE) is the development of tissues able to recapitulate the complex microarchitecture of the native counterpart. The combination of cell sheet (CS) engineering and magnetic force has already demonstrated remarkable outcomes in the development of heterotypic microtissues with enhanced extracellular matrix (ECM) deposition and conservation of tissues' structure (1). Following the same rational, we now propose the combination of a microfabrication-platform with magnetic-TE to generate robust microtissues with complex architecture for TE purposes.

Small free-standing (Fs) tissue units with diverse shapes were designed with high fidelity maintaining their geometry after 7 days post-culturing, overcoming the challenges of microtissues fabrication. The ability of microtissues in invading distinct tissue models while releasing trophic factors was also substantiated in methacryloyl laminarin (LAM) and platelet lysates (PLMA) hydrogels. By simply using cells as building units and such microfabrication-platform, the fabrication of complex multiscale and multifunctional tissues with clinical relevance is envisaged.

METHODS: Magnetic nanoparticles were synthesized and incubated with human adipose-derived stromal cells. After confirming both cell viability and proliferation, the magnetically labeled cells were seeded onto superhydrophobic surfaces patterned with wettable superhydrophilic domains, and then detached with the aid of a magnet, enabling the creation of Fs-CSs. Lastly, the stemness and the ability of the Fs-CS to integrate and invade neighbouring tissue models were evaluated along the time using PLMA and LAM.

RESULTS: Robust microtissues with an enriched collagen-matrix and defined shapes were successfully engineered, demonstrating the ability to create robust microtissues with versatile shape.

Additionally, the Fs-CS inserted in hydrogels-tissue models rapidly sprouted and invaded their structure, showing invasive properties while keeping the stemness features.

DISCUSSION & CONCLUSIONS: The work proposes the high-throughput fabrication of a cell-based microtissue construct, encompassing an array of geometries with high precision and definition, using a straightforward and scalable method.

Such small tissue units can be used as building blocks to fabricate larger, modular and heterogeneous tissue constructs, where each component can be easily adapted to fulfil the requirement of complex and hierarchical tissues. We hypothesize that such platform could endorse the engineering of tissues for regenerative-based therapies with high complexity and heterogeneity or in vitro platforms for disease modelling and drug screening.

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REFERENCES: (1) Silva, A. S. et al Biomaterials, 2019, 119664

Keywords: Biofabrication, Stem cells - general

PP-362

Fabrication of Scaffold-free All-Cellular Living Fibers

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INTRODUCTION: Scaffold-free microtissues have found interest both as tissue models and high retention cell delivery/pro-regenerative devices. Usually, microtissues are assembled as standalone cell sheets and spheroid-shaped aggregates. Both types of tissues are incompatible with free-form deposition and difficult to upscale into high-order 3D structures. Fiber-shaped constructs enable omnidirectional positioning. The conventional fabrication of scaffold-free cellular fibers has required exogenous proteins and sacrificial biomaterials templates. We here report the fabrication of centimeter-long cell-only monotypic and heterotypic fibers, using human adipose-derived mesenchymal stem cell (hASCs) and their combinations with endothelial cells.

METHODS: All-cell fibers comprised hASCs seeded onto wettable channels on superhydrophobic surfaces, generating a hanging column setup after upside-down rotation. The viability and morphometric features of cell fibers were evaluated, as well as their free-form positioning and ability to fill complex-shaped Gel-MA defects. Fibers were also tested for tissue invasion capability and secretory profile of pro-regenerative factors, including HGF, VEGF, Osteopontin, IL-6 and IL-10. In this scope, cellular fibers were either embedded within a liquid Gel-MA followed by UV-mediated solidification, or added to a pre-solidified Gel-MA hydrogel. Monotypic (hASCs) and heterotypic (hASCs+HUVECs) fibers were added separately to chorioallantoic membrane (CAM) ex ovo model, to test their potential for tissue invasion and neo-angiogenesis.

RESULTS: The self-assembly of cells into fiber-shaped constructs rendered highly malleable structures, able to adapt to complex-shaped defects. Those exhibited high viability and homogenous thickness distribution. After 7 days of culture into a Gel-MA hydrogel – here used as a simple model of tissue extracellular matrix -, hASCs fibers were able to integrate the surrounding structure, to sprout and secrete pro-angiogenic (VEGF) and trophic factors (HGF, Osteopontin), as well as immunomodulatory interleukines (IL6 and IL 10).

The integration of cell fibroids into an ex ovo matrix was confirmed, and heterotypic fibers (hASCs+HUVECs) also showed pro-angiogenic properties.

DISCUSSION & CONCLUSIONS: We report the rapid fabrication of all-cellular centimetric fibers in the absence of any externally provided supplement or biomaterial. These micro-to-macro tissues showcasing high viability, tissue integration ability and pro-regenerative secretory profile may find future application in regenerative medicine, or as in vitro screening devices.

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Keywords: Biofabrication, Cell therapy

PP-363

Regenerate rainbow trout skin as food using skin-derived cells and various edible hydrogels

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INTRODUCTION: Cultivated meat has been a popular topic in both academic and industrial research, using animal cells with edible biomaterials to regenerate structured tissues as genuine meat. For seafood realm, cultivated fish is developed with the purpose of eliminating overfishing, microplastic and heavy metal contaminants, and cruelty from massive marine animal slaughtering. Fish filet is one of the ultimate goals that we strive for and consists of mainly muscle tissues incorporated with fat. To even advance the similarity of a piece of fish filet, skin tissue is reconstructed to provide additional visual perception, taste, texture, and nutrients. Moreover, fish skin has been consumed in many countries and cultures throughout the history, hence it should be considered in the cultivated fish production.

METHODS: In this study, rainbow trout skin-derived cells were encapsulated in collagen and gelatin as well as plant-based hydrogels to mimic the stratum compactum in dermis with a comparable thickness.

RESULTS: The distribution, morphology, viability, and specific biomarkers of the cells in the 3D networks were observed using fluorescent staining techniques and laser microscopy, revealing the potential of each biomaterial for skin tissue application. Cell proliferation was measured within 14 culture days using Presto Blue assay, showing differences of cell growth in animal-derived hydrogels versus plant-derived ones. Additionally, mechanical tests including texture analysis were carried out on the cultivated fish skin constructs and compared among different biomaterials.

DISCUSSION & CONCLUSIONS: Overall, this study provides the insights of fish skin in the aspect of food application, especially the biological and mechanical characterizations.

Keywords: Biofabrication, Biomaterials

PP-364

Custom-made poly(ester urethane) tailored for 3D printing by Fused Deposition Modelling

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INTRODUCTION: The interest on 3D printing technology as a fabrication method has grown considerably across many disciplines. 3D printing has, in fact, found application in regenerative medicine as well as in the design of tissue models for personalised medicine, drug development and toxicological testing. The most common 3D printing technology is Fused Deposition Modelling (FDM) which employs a thermoplastic polymer as starting material that is extruded in sequential layers above its melting temperature to create a 3D object. Poly(lactic acid) is the most diffused thermoplastic polymer for FDM in biomedical applications. Other interesting materials are poly(ester-urethane)s (PUs), due to their high chemical versatility which can be exploited to synthesize a plethora of polymers matching the requirements of different applications. For instance, degradable PUs have been extensively studied in cardiac tissue engineering because of their elastomeric properties.

METHODS: With the aim to design a cardiac tissue model, we synthesised a PU from poly(ϵ -caprolactone) diol, 2000Da, 1,4-diisocyanatobutane and L-lysine ethyl ester as chain extender. The PU was characterized by Infrared (IR) spectroscopy, Size Exclusion Chromatography (SEC) and mechanical tests. Thermal characterization was carried out by Thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC) and rheology, to assess polymer suitability for processing in the melt state. Thermal analyses were repeated at different storage times, to evaluate the aging effect. The PU was then microfabricated into scaffolds by melt-extrusion and the characterisation of the extruded polymer was performed to evaluate its stability during the printing process.

RESULTS: Mechanical tests showed an elastic behavior up to ca. 15-20% of strain-Rheological studies estimated a crossover temperature from rubber-like to viscous behavior around 135°C. DSC showed a melting temperature close to 55°C; while thermograms at measured at different storage times evidenced slight variations correlated to the polymer progressive ageing and chain rearrangement.

DISCUSSION & CONCLUSIONS: The PU exhibited proper mechanical behavior to engineer multi-layered structures mimicking the cardiac tissue. A thermal treatment was optimized to undo ageing effects and increase printing process repeatability. The extrusion temperature was set to 120°C to allow a proper viscosity for the printing process and avoid thermal degradation. No polymer degradation was observed in the microfabricated scaffolds, indicating PU suitability for FDM technology.

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Keywords: 3D printing and bioprinting, Cardiovascular

PP-365

The engineering of scaffold-free cartilage using microtissues generated under altered growth factor stimulation regimes

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INTRODUCTION: Scaffold-free tissue engineering strategies using cellular aggregates, microtissues or organoids as 'biological building blocks' could potentially be used for the engineering of scaled-up articular cartilage grafts. However, little is known about how different chondrogenic growth factor stimulation regimes during cellular expansion and differentiation influence the capacity of cellular aggregates or microtissues to fuse and generate functional cartilage. In this study human bone marrow mesenchymal stem cells (MSCs) were additionally stimulated with TGF- β 1 +/- BMP-2 during monolayer expansion and differentiation, and their capacity to undergo chondrogenesis in pellet culture and microtissue format was assessed over 3 weeks of culture.

METHODS: MSCs were expanded in culture under 1) regular expansion media composed of DMEM, P/S, FBS and FGF-2; 2) regular media + TGF- β 1; 3) regular media + TGF- β 1 + BMP-2. For the pellet culture and microtissues system, the regular chondrogenic media (CDM) was prepared as described previously (Burdis et al., 2022) and supplemented with BMP-2.

RESULTS: MSCs displayed a higher proliferative potential during expansion with TGF- β 1 or TGF β 1 + BMP-2. Next, the chondrogenic potential of the human bmMSCs was explored in pellet and in high throughput microtissues systems. After 3 weeks of culture, MSCs stimulated with BMP-2 during expansion and differentiation deposited higher levels of GAGs and collagen in both the pellet and microtissues systems; they also stained negative for calcium deposits. In addition, there was negative staining for collagen X in all the groups. When the fusion capacity of the microtissues was investigated, it was observed that all the groups were completely fused after 24h. After 3 weeks of culture, it was observed that MSCs stimulated with TGF- β 1 during expansion and additionally with BMP-2 during chondrogenic differentiation deposited the highest levels of sGAG. Furthermore, there was negative staining for collagen X in all the groups.

DISCUSSION & CONCLUSIONS: The main findings of this study are: (1) stimulation with TGF- β 1 and TGF β 1 + BMP-2 during monolayer expansion positively impacts the proliferation of human MSCs; (2) in the pellet and microtissues system, superior chondrogenesis was observed following stimulation with BMP-2 during both the expansion and differentiation phases; (3) microtissues maintain their fusion capacity in all cases. This study demonstrates the importance of carefully optimizing MSC expansion and differentiation conditions when developing modular tissue engineering strategies using cellular aggregates or microtissues.

REFERENCES: Burdis, R., et al. Biomaterials.

Keywords: Biofabrication, Biologics and growth factors

PP-366

3D bioprinted gellan gum/laponite vessel-like structures for endothelial tissue engineering

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INTRODUCTION: 3D bioprinting of hydrogel scaffolds is essential in tissue engineering (TE) allowing the manufacturing of custom-designed implants which can be used to produce constructs based on patients' needs [1]. Freeform reversible embedding of suspended hydrogels (FRESH) bioprinting provides a solution with good spatial resolution for low viscosity and slow polymerizing biomaterials. Gellan gum (GG) and laponite (Lp) were blended as ink mixtures for the FRESH bioprinting. GG is a natural biocompatible polysaccharide, which possesses angiogenic properties [2]. The addition of Lp to GG improves the printability, increases the pore size favoring the formation of vascular network [3]. The biofabricated vessel-like structures containing L929 fibroblasts have been evaluated for their mechanical stability, swelling properties and in vitro biocompatibility.

METHODS: Two laponite suspensions (0.5% and 1% w/v) were prepared by stirring for 12 h at RT. Then, 1% w/v GG powder was added under stirring. Both Lp/GG inks were combined with L929 cells (3×10^6 cells/bioink ml), and were bioprinted in hollow tubular structures. A 5% w/v gelatin support bath was prepared at 37°C. The produced tubular structures were crosslinked with KCl. Optimal printing parameters were selected to achieve best shape fidelity. The swelling properties of the bioinks have been determined and their Young modulus has been evaluated. For their biocompatibility evaluation, the constructs were stained with the live/dead reagents and visualized under a confocal laser fluorescence microscope.

RESULTS: The average height of the tubular constructs was 1 cm with an inner diameter of 1 mm similarly to medium arteries structure [4]. Both Lp-GG compositions, the 0.5%Lp-GG and the 1%Lp-GG indicated high cell viability of 84 and 88% on day 3, and Young modulus values of 10 and 12 MPa, respectively, matching the characteristics of the native femoral and internal mammary arteries [5]. Increasing the Lp concentration led to a non-significant decrease of swelling ratio from 94 to 91%.

DISCUSSION & CONCLUSIONS: Cell-laden Lp-GG bioinks were used to create hollow tubes mimicking the features of blood vessels. Both bioinks maintained high shape fidelity compared to the respective CAD designs, high viability and elasticity and enhanced swelling properties. Ongoing investigation includes their endothelial regeneration capacity.

ACKNOWLEDGEMENTS: This research was funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) project number HFRI- FM17-1999.

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Keywords: Biofabrication, 3D printing and bioprinting

PP-367

Antibacterial and MRI-visible Scaffolds out of Metal-Organic Framework / Polycaprolactone for Tissue Engineering Applications by Melt Electrowriting

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INTRODUCTION: Melt electrowriting (MEW) is an additive manufacturing technique that allows the creation of defined microarchitectures of fibrous scaffolds with applications in tissue engineering. However, the technique possesses a limited material library. To further expand the potential of MEW and to facilitate the translation of melt electrowritten implants towards the clinic, this study presents the use of silver-loaded, iron-based metal-organic frameworks (MOF) embedded in polycaprolactone (PCL) to obtain multifunctional scaffolds with antibacterial properties and magnetic resonance imaging (MRI)-visibility using melt electrowriting.

METHODS: Scaffolds of PCL/MOF composites with different percentages of MOF were melt electrowritten. The pressure, voltage and the speed between the needle and the collector were optimized for the different MOF concentrations. The morphology of the fibrous samples was analyzed by SEM. The antibacterial efficacy was evaluated for the strains *S. epidermidis*, *S. aureus* and *E. coli* by plating serial dilutions of bacterial suspensions on incubated samples and counting colony forming units (CFU). The melt-electrowritten samples were tested for cytocompatibility according to the indirect test in ISO 10993, where, after 72 hours incubation, the sample eluates were transferred onto smooth muscle cells and cell viability determined at day 3. Pure PCL and PCL/MOF meshes were visualized with a 7 T magnet and 10 mm RF solenoid coil. T1-T2*w images, and R2, R2*, and R1 maps were acquired.

RESULTS: Scaffolds with up to 20% MOF were successfully printed, with a fiber diameter of 50 µm for all samples. 5% MOF loading was found to be the optimal concentration, as the resulting MEW scaffolds exhibited antibacterial efficacy against *S. aureus*, *E. coli* and *S. epidermidis* with an average reduction of 99.5%, while maintaining the PCL cytocompatibility according to the ISO 10993. These PCL/MOF samples also showed ten-fold increase in the R2* relaxation rate compared to the pure PCL samples, thereby serving as MRI contrast agents.

DISCUSSION & CONCLUSIONS: This study shows great potential for manufacturing multifunctional microfibrous scaffolds for tissue engineering by combining MEW with MOFs. Here, silver serves as an antibacterial agent that does not further the growing antibiotic resistance, whereas iron enables visualization with a clinically accepted monitoring method. Although demonstrated here with MOFs loaded with silver nanoparticles, the exploitation of MOFs as additives and carriers opens up the exciting possibility to design complex multifunctional scaffolds, with applications such as drug delivery, imaging, biosensing.

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Keywords: Additive manufacturing, Biofabrication

PP-368

Novel biofabrication approaches enabled by di-tyrosine crosslinking of silk fibroin

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INTRODUCTION: Covalently crosslinked silk fibroin hydrogels have gained popularity over their physically crosslinked (via beta-sheet formation) counterparts due to their elastomeric nature, transparency and ability to support cell encapsulation, as well as compatibility with modern biofabrication approaches. Covalent crosslinking is achieved via di-tyrosine bond formation between tyrosines natively found in silk, without the need for any modification of the polymer chain. This reaction can be mediated through three broad strategies, including enzymatic-, Fenton reaction-, and photo-initiated crosslinking approaches.

METHODS: This talk will discuss the broad utility of ruthenium-based photo-crosslinking of silk for multiple biofabrication approaches and material formats, including hydrogels, microgels, granular hydrogels and lyogels.

RESULTS: We demonstrate the formation of rapidly crosslinking hydrogels at concentrations as low as 1% wt/v silk with tuneable compressive modulus (~1-100 kPa) and excellent optical properties (transmittance >90%). These hydrogels can be fabricated into complex high-resolution structures via sacrificial polymer templating or ice templating. Ice templating allowed formation of aligned pores and incorporation of complex structures such as channels, multiple material phases and drug encapsulation in large multi-centimeter scale constructs. Water-in-oil emulsion within a flow-focusing microfluidic device allowed the formation of small (~98±18 µm) or large (~375±28 µm) silk microgels which when annealed together resulted in scaffolds with microporosity and supported excellent cell encapsulation, proliferation and tissue ingrowth in vivo relative to their bulk hydrogel counterparts.

DISCUSSION & CONCLUSIONS: This work offers new silk biomaterial platforms for biomedical applications and novel insights into di-tyrosine bond formation and the dynamic nature of silk crosslinking.

Keywords: Biofabrication, Biomaterials

PP-369

Development of a nanocomposite bioink library for the 3D bioprinting of skeletal tissue to aid bone repair

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INTRODUCTION: Nanoclays are a versatile family of biocompatible and bioactive nanomaterials. Indeed, nanoclays can: (i) assemble and gelate rapidly in the presence of ions, (ii) demonstrate shear-thinning properties in suspension in an aqueous solvent and, (iii) the capacity to retain and localise encapsulated compounds [1]. Herein, we explore a number of nanocomposites (comprising alginate, methylcellulose, gellan gum, GelMA) for bone regeneration. The current studies demonstrate the ability of Laponite® (LAP) nanoclay to support the 3D bioprinting of skeletal implants and stimulate the formation of bone tissue in vitro and ex vivo. Specifically, we demonstrate the use of human bone decellularised and demineralised extracellular matrix (hbECM) in combination with nanoclay filler for skeletal tissue repair.

METHODS: hbECM was prepared from donated bone tissue. The mineral and cellular fractions were removed following a series of washes with 2%P/S followed by digestion in 0.5 N HCl with trypsin/EDTA. Silicate nanoclay, namely Laponite® (LAP, BYK, UK) was blended with alginate to facilitate ink printability. Material inks were tested for physical properties (SEM, mass loss, swelling ratio), print fidelity and the ability to support HBMSCs viability (live-dead) and phenotype (alkaline phosphatase (ALP) staining). Ex vivo studies examined HBMSC-laden and cell-free controls in 3D scaffolds in the chicken chorioallantoic membrane (CAM) model for 7 days following incorporation of vascular endothelial growth factor (VEGF) or bone morphogenetic protein 2 (BMP-2) to stimulate angiogenesis and osteogenesis.

RESULTS: Nanocomposite inks exhibited negligible swelling behaviour compared to the nanoclay-free material, while demonstrating higher viscoelastic properties ideal for 3D printing. Acellular material inks were tested for printability, identifying the nanocomposite as suitable for cell printing. HBMSCs were printed at 106 cell ml⁻¹ and cultured up to 21 days, demonstrating a higher proliferation rate ($p < 0.001$) compared to the nanoclay-free controls. The presence of nanoclay did not affect cell viability allowing the sustained retention of cells within the printed hydrogel structure up to 21 days with evidence of proliferation compared to LAP-free controls. CAM implantation revealed that the inclusion of nanoclay with alginate and bone ECM facilitated the sustained release of VEGF, promoting a vasculature network significantly larger ($p < 0.001$) compared to VEGF-free and LAP-free controls.

DISCUSSION & CONCLUSIONS: The current studies demonstrate the synergistic combination of nanoclay and biomimetic materials, (alginate and hbECM) support the formation of osteogenic tissue both in vitro and ex vivo and offer promising novel 3D bioprint approach to repair skeletal tissue.

REFERENCES: [1] Cidonio G et al. Biofabrication 12:035010, 2020

Keywords: Biofabrication, Bone and bone disorders (osteoporosis etc)

PP-370

Integration of Computational Modelling and Biofabrication Technologies for Bone Tissue Engineering

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INTRODUCTION: This work presents an innovative approach to design three-dimensional (3D) scaffolds for the healing of osteoporotic bone fracture integrating computational modelling and biofabrication technologies. The scaffold is fabricated by Fused Deposition Modelling (FDM) printing of PLLA-based filaments loaded with inorganic phases, and then functionalised with a bioactive protein. The study is focused on the scaffold design through computational modelling, and on the definition of a biofabrication protocol. Finally, a mock-up of a long bone model was 3D printed as a proof-of-concept.

METHODS: A CAD model of the scaffold was ad hoc designed starting from CT scans of a long bone. Then, the geometry was optimized by implementing a hydrolytic degradation model [2] to achieve a time-/space-controlled delivery of the grafted protein towards the fracture.

Subsequently, a biofabrication protocol was defined to print the scaffold with PLLA-based blend filaments loaded with Strontium substituted nano-hydroxyapatite, selected among different formulations by in vitro testing cylindrical scaffolds.

A previously designed bioprinting platform [3] was optimized to control the printing environment (e.g., temperature, air quality) with specific sensors. The printing parameters were adjusted to improve the scaffold's mechanical properties and surface quality. As a proof-of-concept, the long bone model was 3D printed in PLA to demonstrate the feasibility of placing the scaffold with the supposed fixation mechanisms (i.e., metallic plates, screws, and cerclage wires).

RESULTS: By evaluating the release kinetic of the grafted protein, the optimized geometry was selected for the biofabrication. The scaffold was then successfully fabricated with the bioprinting platform using the PLLA-based filament, whose in vitro tests showed good biocompatibility and an increased osteogenic potential due to the presence of the nano-hydroxyapatite substituted with Strontium. The scaffold was fixed on the 3D-printed long bone model, showing perfect contact to the surface, and stable placement with the fixation mechanisms.

DISCUSSION & CONCLUSIONS: This work presented a novel approach for designing and fabricating scaffolds for osteoporotic bone fractures, which will be validated through in vivo testing. As a future development, the computational model will also account for the effect of the degradation on the mechanical properties.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No814410.

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Keywords: Biofabrication, Bone and bone disorders (osteoporosis etc)

PP-371

Near-field direct-writing electrospinning of architectures made by nature-derived polymers for tissue engineering

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INTRODUCTION: If compared to Fused Deposition Modeling, near-field direct-writing electrospinning (NFES) allows to precisely deposit a thinner filament and, therefore, to more accurately replicate tissue architectures. Nevertheless, NFES use is still limited to process natural polymers for regenerative medicine.

This study aims at fabricating via NFES well-defined architectures with submicrometric fibers using nature-derived polymers not yet processed with NFES.

METHODS: Gelatin B and Bovine Serum Albumin (BSA) were dissolved 10:1 in 70% v/v acetic acid, testing three different concentrations (22, 27.5 and 45% w/v). A custom-assembled NFES setup with a user-friendly XY moving collector (speed: 8000 mm/min; acceleration: 1500 mm/s²) was used to arrange single fibers in parallel arrays (20x20 mm²) or circles ($\phi=26$ mm). Effect of electric field (1 or 1.5 kV/mm) and, for parallel arrays, of fiber spacing (0 or 500 μ m) was investigated.

After process optimization, insoluble scaffolds were prepared using Methacrylated Gelatin and BSA: multi-layered parallel fiber arrays (10x10 mm²) were iteratively deposited increasing fiber spacing in progress. Three sample configurations were obtained performing different overall process iterations (300, 600 and 900) and then UV photocrosslinked.

For both single fibers and multi-layered samples, fiber morphology was evaluated by SEM and acquired images were analyzed using ImageJ software to measure mean fiber diameter and alignment degree.

RESULTS: With 22% w/v concentration, jet formation was ensured over a wide range of environmental conditions (T=20-26°C, R.H.=26-49%) and both continuous fiber deposition and process stability were allowed thanks to an optimized combination of operational parameters. Single parallel fibers were submicrometric at 1.5 kV/mm whereas alignment and straightness were maintained even at 1 kV/mm, but narrower spacings and higher electric fields led to more coiled fibers. At 1.5 kV/mm, submicrometric circular fibers could be accurately deposited without coils apart from some confined regions.

In multi-layered samples, alignment degree of almost 70% was reached, but it decreased to 45% beyond 900 iterations probably for electric field alterations by stacked fibers. Moreover, 83% of fiber diameters were suitable for anisotropic tissues regeneration.

DISCUSSION & CONCLUSIONS: Submicrometric fibers were easily oriented along various trajectories via NFES with reproducible morphological features, comparable to extracellular matrix dimensions (~ 0.5 μ m). Control over fiber deposition was exerted even at motion direction changes, although higher collector accelerations may restrict coiled traits.

Versatility and precision demonstrated by the developed system allow to design complex and even multi-layered architectures made by biocompatible nature-derived polymers. Future work will address biological assessment of the so-obtained structures.

Keywords: Fibre technology, Polymers - natural / synthetic / responsive

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A digital reconstruction of the dermal-epidermal junction (DEJ) of the skin in three dimensions

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INTRODUCTION: The dermal-epidermal junction (DEJ) is a basement membrane which separates the dermis and epidermis within the human skin. The DEJ has a unique topography, its surface undulates and forms a dense interconnecting collagen network. The DEJ has not been studied fully, but it has been shown to maintain skin integrity as well as assist in skin homeostasis, layer adhesion, and function. Tissue engineering (TE) allowed the development of skin regeneration strategies and TE scaffolds for clinical use. However, the currently available TE skin equivalents neglect to replicate the DEJ anatomical structures. This study explores the digital reconstruction of the DEJ via computer-aided design (CAD) to form 3D models. 3D models were produced using additive manufacturing. The main aim is to develop 3D models which mimic the DEJ topography at a cellular level. Here, we present high-resolution 3D CAD and 3D printed models with different topographies, cuboidal and sinusoidal waveforms. Another 3D model was created using a stack of optical coherence tomography (OCT) images of the isolated DEJ to further assess the effectiveness of topography and dimensions of each model on cells during cell culture.

METHODS: STL files were created successfully by Autodesk Fusion 360. The dimensions of both pattern types were modelled to match the range of dimensions in the DEJ and one measurement beyond this range. Amira software was used to output 3D models of the human DEJ.

RESULTS: 10 unique 3D models have been designed and produced, hence the effect of geometries and dimensions on cellular behaviour is investigating. Due to micron range limitation, a high-resolution 3D printer is required to enable to print of the patterns with high resolution. In the beginning, the Form2 SLA printer was used with a layer resolution of 25 µm; however, due to the laser spot-size of 140 µm this printer struggled to output greater print quality.

DISCUSSION & CONCLUSIONS: This research supports the importance of a corrugated structure and the clinical relevance of DEJ design, for adoption in skin tissue engineering to replace full-thickness skin defects or alternatively modelling wound healing, pathological conditions and producing and screening drugs.

ACKNOWLEDGEMENTS: Thanks to Prof. Stephen J Matcher for providing the OCT images used in this study.

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Keywords: 3D printing and bioprinting, Additive manufacturing

PP-373

Multi-scale tubular Melt-electrowritten PCL constructs for small- to medium-sized prosthetic vascular grafts

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INTRODUCTION: Blood vessels are the transportation system for blood and nutrients. A common pathology in a blood vessel involves a narrowing or total occlusion(Mallis et al. 2020), often requiring a vascular graft for treatment. Small-diameter prosthetic vascular grafts remain a challenge, because they largely remain without an endothelium(Zilla et al. 2007), potentially due to sub-optimal mechanical properties or surface characteristics. Therefore, new viable artificial vascular grafts with appropriate biomechanical and biological properties are needed to improve treatment options. Melt-electrowriting(MEW) represents a promising biofabrication tool for the creation of vascular grafts, as it allows to precisely control the deposition of micro-diameter polymer fibres, providing better control of the structure and therefore local mechanical properties.

The aim of this study was to characterize the influence of an inner layer, imitating the Tunica intima, fabricated in two ways, on the overall structural properties of tubular graft-like structures formed with a microfiber grid MEW layer as the main structural component.

METHODS: Three types of tubular scaffolds were produced using an in-house developed MEW setup, in conjunction with conventional solution-electrospinning(SES). Group1 was a MEW microfiber layered grid alone, Group2 was the same grid with an added thin SES membrane on the inside while Group3 had a thinner fiber MEW membrane with different fiber orientations on the inside of the MEW microfiber grid.

Fiber diameter and scaffold wall thickness was assessed using scanning electron microscopy and optical profilometry. Mechanical evaluation was done under quasi-static conditions as a tensile test in the longitudinal and circumferential directions of the tubular scaffold.

RESULTS: The scaffold wall thickness was 188.3µm(SD=21.8µm), 208.0µm(SD=21.8µm) and 228.1µm(SD=22.7µm) for Group1, Group2 and Group3, respectively. The yield stresses observed in the longitudinal and the circumferential testing direction were 0.23MPa and 0.26Pa, 0.34Pa and 0.24MPa, and 0.41MPa and 0.27MPa for Group1, Group2 and Group3, respectively.

DISCUSSION & CONCLUSIONS: The inner layer on the MEW tubular scaffold changes the behavior of the scaffold under tension at low strains. The multiscale scaffolds achieve higher stress values, especially with a SES inner layer, where the maximum stress was almost doubled for the longitudinal direction. In the circumferential direction, no substantial improvement was observed in yield stress values. However, the scaffold response was different in the toe and linear regions. While the results indicate that an inner layer can improve the mechanical characteristics of a MEW scaffold, the mechanical properties are still an order of magnitude lower of that of native vascular tissue and need further development.

Keywords: Cardiovascular, Fibre technology

PP-374

Digital light processing of gelatin-based biomaterial inks towards superior in vitro intestinal models

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INTRODUCTION: In 2017, the second most commonly diagnosed cancer type in Belgium was colorectal cancer. To gain superior understanding of the disease, in vitro intestinal models with physiological mimicry are necessary. Moreover, these models can be exploited for the evaluation of the nutritional value of food supplements and the efficiency of novel drugs.

The model requires a construct with sufficient permeability towards nutrients and drugs (equilibrium permeability within 24 hours), whilst obtaining suitable stiffness ($G' < 10$ kPa) and a close mimic of the intestinal architecture. Therefore, a gelatin-methacryloyl-aminoethyl-methacrylate (gel-MA-AEMA)-based biomaterial ink was exploited for digital light processing (DLP).

METHODS: Characterization of 2D gel-MA-AEMA-based hydrogel constructs

To assess the stiffness of the hydrogel constructs, oscillatory rheology measurements were carried out on 200 μ m thick, swollen hydrogel discs at 37°C. A static diffusion setup was exploited to determine the permeability of the hydrogels towards FITC-dextran 4 kg/mol.

Development of gel-MA-AEMA-based biomaterial ink

To determine the optimal biomaterial ink composition, in situ rheological evaluation of the formulations was implemented. The most promising inks were subsequently tested for their printing potential on a LumenX+ (CellInk) 3D printer.

Morphological evaluation of the hydrogel constructs

To evaluate the morphology of the 3D printed hydrogel constructs, optical microscopy and cryo scanning electron microscopy have been utilized.

In vitro biocompatibility assay of the hydrogel constructs

A live-dead staining (calcein-AM, propidium iodide) of seeded Caco2 and HT29-MTX cells in co-culture (9:1) was performed.

RESULTS: Mechanical evaluation and permeability properties of the 2D hydrogel constructs

The mechanical properties of the hydrogel constructs were adequate ($G' = 1.56 \pm 0.63$ kPa). Within 24-hours, $40.5 \pm 2.6\%$ of FITC-dextran 4 kg/mol diffused to the basolateral compartment, while $24.8 \pm 2.0\%$ was retained in the apical.

Morphological and biological evaluation of the 3D printed hydrogel constructs

The morphological analysis of the printed constructs showed that physiologically relevant villi-like structures were successfully 3D printed, showing excellent biocompatibility towards a co-culture of Caco2 and HT29-MTX cells (ISO 10993-5-2009).

DISCUSSION & CONCLUSIONS: A gel-MA-AEMA-based biomaterial ink was successfully developed for

DLP 3D printing processed towards a 3D villi-like soft hydrogel scaffold and combined with a co-culture of Caco2 and HT29-MTX cells indicating the potential of the model.

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Keywords: 3D printing and bioprinting, Polymers - natural / synthetic / responsive

PP-375

Development of melt electrowritten scaffolds coated with mesoporous bioactive glass nanoparticles (MBGNs)-laden gelatin for biomedical applications

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INTRODUCTION: Melt electrowriting (MEW) is an electrohydrodynamic-based additive manufacturing technique using solvent-free processing to print fibers at the micron scale [1]. Recently, researchers have focused on MEW scaffolds for biomedical applications because they exhibit structures mimicking the extracellular matrix's biological and mechanical properties with adequate pore size and interconnected porous structure [1,2]. In particular, polycaprolactone (PCL) has been widely used for MEW due to being a thermoplastic with a low melting point, whereas the cell adhesion and proliferation responses to PCL are limited [1]. Therefore, coating PCL MEW scaffolds with natural polymer gelatin (GEL) should promote cell adhesion and proliferation properties. On the other hand, mesoporous bioactive glass nanoparticles (MBGNs) have drawn attention in biomedical applications because of their unique physical, chemical, and biocompatibility properties [3].

METHODS: The current study aims to develop innovative strategies to fabricate PCL-MEW scaffolds coated with MBGN-laden GEL solution for biomedical applications. In this regard, PCL scaffolds were first fabricated according to the MEW method and subsequently treated with alkaline surface modification to improve the coating of the MBGN containing GEL layer. Lastly, the scaffolds were dip-coated into the MBGN-incorporated GEL solution.

RESULTS: Scanning electron microscopy images revealed that the alkaline surface modification did not cause morphological defects on PCL-MEW scaffolds. Additionally, the results showed that MBGNs were uniform and less aggregated. The presence of PCL, GEL, and MBGNs was confirmed using Fourier transform infrared spectroscopy. Moreover, the biological activity of samples was tested using normal human dermal fibroblast (NHDF) cells. The biocompatibility results demonstrated that samples have no cytotoxic effect on NHDF cell lines. Additionally, alkaline surface modification, as well as the MBGN-laden GEL layer, enhanced cell viability. Furthermore, the evaluation of cell morphology using SEM images, Calcein/DAPI, and rhodamine-phalloidin/DAPI staining confirmed that the MEW scaffolds coated with MBGN-laden GEL layer do not exhibit cytotoxicity and do not lead to cellular abnormality.

DISCUSSION & CONCLUSIONS: Consequently, the findings imply that the developed PCL-based MEW scaffolds are promising for biomedical applications.

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Keywords: Additive manufacturing, Biomaterials

PP-376

Synthetic and semisynthetic extracellular matrix mimics for patient-derived breast cancer organoid culture enable translational precision medicine workflows

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INTRODUCTION: Advancements in 3D organoid technology have resulted in the emergence of cutting-edge precision medicine tools for cancer. However, the commonly employed gold standard 3D cell culture system Matrigel® exhibits significant limitations, including the incapacity for substantial biophysical manipulation, presence of undefined growth factors, and extensive batch-to-batch variability. Tuneable hydrogel matrices demonstrate enhanced potential for drug testing in breast cancer (BCa) due to their improved mimicry of the physicochemical characteristics of the native extracellular matrix in healthy and diseased states.

METHODS: This study analyzed the formation and therapeutic response of patient-derived BCa organoids in bioprinted polyethylene glycol- (PEG) and gelatin-based hydrogels of varying crosslinking chemistry (gelatin methacryloyl; GelMA and thiolated-gelatin crosslinked with PEG-4MAL; Gel-SH;

Gelomics supplied by Gelomics). Briefly, surgically-resected tumor specimens were collected from consenting donors and enzymatically digested to recover cells. Following monolayer cell expansion, cells were encapsulated in drop-on-demand bioprinted PEG-based matrices (+/- RGD, GFOGER and DYIGSR; RASTRUM, Inventia Life Science), photocrosslinkable (GelMA) or click-crosslinkable gelatin (Gel-SH), and organoid formation as well as chemotherapy treatment response was evaluated.

RESULTS: Our results indicated that GelMA, Gel-SH, and PEG-based hydrogels effectively supported the growth and organoid formation of HR+,–/HER2+,– primary cancer cells within a range of BCa extracellular matrix stiffnesses (1–6 kPa) for a minimum of 2 to 3 weeks, with superior organoid formation observed in Gel-SH (up to 268% growth after 15 days). BCa organoids demonstrated increased IC50 concentrations in response to doxorubicin, EP31670, and paclitaxel treatments compared to 2D cultures, with the highest IC50 recorded for the well-established organoids in Gel-SH. Doxorubicin treatment (1 μ M) resulted in cell viabilities that were 2-fold higher in the 3D gels compared to 2D, and doxorubicin/paclitaxel (both 5 μ M) were 2.75 to 3-fold less potent in Gel-SH compared to PEG hydrogels.

DISCUSSION & CONCLUSIONS: The results of this study indicate that PEG- and particularly click gelatin-based hydrogel matrices serve as an economical, tuneable, consistent, and translatable cell culture system for patient-derived BCa organoids, surpassing the limitation of the gold standard culture system, Matrigel®.

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Keywords: Biomaterials, In vitro microenvironments

PP-377

A versatile organ-on-a-chip model for the evaluation of proangiogenic biomaterials

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INTRODUCTION: Organ-on-a-chip technologies have become an important research tool in the last decade. It is now possible to create cellular microenvironments that mimic tissues in a controlled manner to study the effects of various stimuli, signals, processes, and drugs. Here, we have developed a compact microfluidic platform as an analog to study and model the effect of different microenvironment conditions on blood proto-vessels genesis.

METHODS: After *in silico* optimization through finite element simulations of flows, diffusion of species, and signal gradient through cells' hosting fibrin hydrogel, we fabricated the platforms using CAD software and soft lithography in PDMS. We used fluorochrome analogs to validate experimentally the computer models [1]. The materials to be tested were prepared by electrospinning polylactic acid as the main component and adding calcium-releasing nanoparticles to trigger the expression of proangiogenic factors in cells. Rat endothelial progenitor cells (rEPCs) were tested in devices with or without the proangiogenic biomaterial. Immunohistochemistry was used in conjunction with confocal fluorescence imaging to contrast the cellular cytoskeleton and nuclei and measure material efficacy and cell migration. To study gene and protein expressions of proangiogenic biomolecules, we used ELISAs and RT-qPCRs. A dynamic *in silico* phase field model of migration used the results of the migration assay as boundary conditions [2].

RESULTS: Several intriguing, sometimes unexpected, facts emerged from experimental and *in silico* RESULTS: the angiogenesis platform proved useful for assessing proangiogenic biomaterials. Gradients of calcium ions promote the release of vascular-promoting signaling from mesenchymal stem cells. rEPCs had to degrade the local ECM to migrate more quickly, and the size of the ECM pore had a significant impact on the proliferation of sprouting tips in proto-vascular growth

DISCUSSION & CONCLUSIONS: OoCs offer viable alternatives to *in vitro* and *in vivo* characterization techniques. To model physiological processes like angiogenesis, we have also shown that is a viable option for data mining. They can then be used to develop powerful deep-learning algorithms. OoCs are a game-changing technology that might potentially be more adaptive and efficient than the alternatives we now have. Finally, more research is required, notably in the control of the hosting hydrogel's 3D porosity, viscoelastic properties, and degradability, to entirely control experimental conditions and provide more accurate correlations.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Vascular systems / vascularisation and heart

PP-378

Modeling hyperglycemia using a vessel-on-a-chip technology and endothelial progenitor cells

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INTRODUCTION: Studying hyperglycemia-induced vascular dysfunction and methods to restore the endothelium are important aims in diabetes. This is better replicated in a 3D-format that mimics the physiological structure of the vessel and incorporates cell-cell and cell-matrix interaction. Organ-on-a-chip(OOAC) allows drug testing on organ micro-models developed using human cells under microphysiological conditions, thus maintaining human genetic background under complex physiological settings. A potential cell source to screen drugs using OOAC is blood endothelial progenitor cells(EPCs), which are easy to isolate with high proliferative capacity, providing an autologous source for personalized drug testing. The aim of the current work is to (i)develop 3D vessels using human blood outgrowth endothelial cells (BOECs) expanded from EPCs and study hyperglycemia effects on vessel formation and function in an OOAC model, (ii)test the effect of the antidiabetic drug "metformin" and the endothelial nitric oxide synthase (eNOS)-restoring drug "sildenafil" on vessel proliferation and function.

METHODS: BOECs were isolated from healthy donors using selective plating¹. To develop 3D vessels, an OrganoPlate® 2-lane system(MIMTAS) was used. Neutralised Type I collagen(4mg/ml) was seeded into the gel channel and was polymerized for 15 min, followed by seeding of pre-stained BOECs (20,000cells/well) into the perfusion channel. Plates were left standing at 75°angle to allow cell adhesion to the gel layer, and then incubated on a rocker to allow media perfusion. Vessels were cultured in normal(5.5mM) or high glucose(25mM) EGM-2 media for 72hr in the presence or absence of metformin(50uM) or sildenafil(10uM). Vessel formation was examined by confocal imaging using z-stacks, and viability was assessed using alamarBlue proliferation assay. Barrier-function was assessed by tracking the leakage of fluorescently-tagged-dextran from the vessel to the gel channel.

RESULTS: BOECs were able to form 3D vessels under both normoglycemic and hyperglycemic conditions, with slower formation under hyperglycemic conditions. This was also reflected in vessels' viability which was reduced under hyperglycemic conditions at 72hr. Treatments with metformin and sildenafil for 72hr increased cell proliferation in both conditions(n=4). Our preliminary data showed an increased permeability to 20KDa FITC- dextran in vessels chronically exposed to hyperglycemia, with sildenafil reducing permeability under these conditions.

DISCUSSION & CONCLUSIONS: Herein we have modeled hyperglycemia using 3D vessel-on-a-chip made of EPCs. The responses of the vessel to hyperglycemia in terms of proliferation and permeability reflected the current knowledge about vascular endothelium.

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Keywords: Disease models, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-379

Robust Human Organoid Printing and Culture on a Pillar/Perfusion Plate Platform for Predictive Compound Screening

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INTRODUCTION: There have been several three-dimensional (3D) cell culture platforms developed, including ultra-low attachment well plates, Transwell inserts, hanging droplet plates, and microfluidic plates, but these platforms are relatively low throughput and/or unsuitable for high-throughput organoid culture and analysis in situ.

METHODS: To facilitate robust organoid culture in a high-throughput screening (HTS) system, we have developed “microarray 3D bioprinting” technology and a pillar plate and a complementary perfusion well plate, which is highly flexible, user-friendly, and easily combined with conventional 384-well plates to support organotypic cell cultures and multiplexed high-content imaging assays.

RESULTS: We have demonstrated that our microarray 3D bioprinting platform can be used for reproducible and scale-up generation of human organoids that are critical to successfully perform predictive compound screening with organoids. Several human organoids including brain, liver, liver tumor, and intestine have been successfully printed, encapsulated in biomimetic hydrogels including Matrigel, BME2, and alginate, differentiated, and imaged on the pillar/perfusion plate platform for high-throughput compound screening. Interestingly, dynamic culture of human organoids on the pillar/perfusion plate significantly enhanced the maturity of human organoids potentially due to rapid diffusion of growth factors and small molecules necessary for cell differentiation and maturation. The optically clear pillar/perfusion plates allowed direct visualization of organoids on the pillars for predictive cell-based assays. The entire organoids on the pillar plate were permeabilized, fixed, stained with primary and secondary antibodies, and cleared with tissue clearing solutions simultaneously for in situ whole organoid imaging without the need for cryosectioning. The flexible pillar and perfusion well format connected by microchannels and reservoirs made it easy to change growth media for organoid culture without the use of bulky pumps and tubes. It is compatible with standard 384-well plates and existing HTS equipment including fluorescence cell imagers and microtiter well plate readers, which is an important feature for developing HTS assays. It is easy to connect different types of organoids cultured on the pillars by using the perfusion well plate, which is critical to simulate human diseases.

DISCUSSION & CONCLUSIONS: Thus, our microarray 3D bioprinting technology and the unique pillar/perfusion plate platform could offer new opportunities for creating highly organized organoids by dispensing human pluripotent stem cells in hydrogels precisely with printing robots and mimicking the microenvironment of tissues in vivo, thereby potentially revolutionizing regenerative medicine, oncology, and drug discovery.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, 3D printing and bioprinting

PP-380

Enhanced cell growth, reproducibility, throughput, and user friendliness in dynamic 3D cell culture using a pillar/perfusion plate platform

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INTRODUCTION: Several three-dimensional (3D) cell culture platforms have been developed, including ultra-low attachment (ULA) well plates, Transwell inserts, hanging droplet plates, and microfluidic devices. However, these platforms are relatively low throughput, user unfriendly, and/or unsuitable for high-throughput, dynamic 3D cell culture and analysis in situ.

METHODS: To facilitate robust, dynamic 3D cell culture in a high-throughput screening (HTS) system, we have developed a pillar/perfusion plate, which is highly flexible, user-friendly, and easily combined with conventional 384-well plates to support dynamic 3D cell cultures and multiplexed high-content imaging assays.

RESULTS: To demonstrate the proof of concept, we generated Hep3B hepatoma cell spheroids in a ULA 384-well plate, which were transferred to the pillar plate by simple sandwiching and encapsulation in Matrigel. Hep3B cell spheroids generated on the pillar plate as well as in the ULA plate as a control were cultured in static and dynamic conditions to compare their cell growth and viability. Both spheroids cultured in the ULA plate in a static condition and on the pillar/perfusion plate in a dynamic condition showed similarity in size. The coefficient of variation obtained was below 20%, indicating high reproducibility of the spheroid transfer. The flexible pillar/perfusion well format connected by microchannels and reservoirs made it easy to change growth media for dynamic cell culture without the use of bulky pumps and tubes. It was compatible with fluorescence cell imagers and microtiter well plate readers for HTS assays. Interestingly, there were many dead cells observed in the core of Hep3B spheroids cultured in the ULA plate due to diffusion limitation whereas dynamic spheroid culture on the pillar/perfusion plate significantly enhanced cell survival/growth by preventing cell death in the core. The viability of Hep3B spheroids in the ULA plate was 2-fold lower despite its large size. We confirmed that there was no hypoxia in the core of cell spheroids measured by Image-iT hypoxia detection reagent. In addition, qPCR analysis confirmed that the expression levels of anti-apoptotic markers were significantly elevated in dynamic-cultured spheroids while downregulating those of pro-apoptotic markers.

DISCUSSION & CONCLUSIONS: Our unique pillar/perfusion plate could be used for high-throughput, dynamic 3D cell culture by loading cells in hydrogels precisely with printing robots or by manual stamping. Dynamic 3D cell culture could potentially recapitulate tissue function in vivo, thereby enhancing predictivity of compound screening.

ACKNOWLEDGEMENTS: This study is supported by the National Institutes of Health (NIEHS R01ES025779, NIDDK UH3DK119982, and NCATS R44TR003491).

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, 3D printing and bioprinting

PP-381

Endothelialization of organ-on-chip models for increased biological relevance on a high-throughput platform

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INTRODUCTION: To study both intrusion of harmful agents and access of therapeutic ones, microfluidic organ-on-chip models have been developed to replace the current standard 2D cell culture models lacking biological relevance. These new generation models are also potent alternatives for animal experimentation. However, current organ-on-chip models suffer from low throughput which hampers the test of numerous biologicals. Here, we present a standardised, high-throughput platform termed AKITA® Plate, which allows establishment of up to 96 barrier cultures on one SBS standard plate. This microfluidic plate is easy to handle, compatible for automatized confocal imaging and is able for seamless integration to industrial robotic handling systems.

METHODS: Endothelialized cultures on AKITA® Plate are grown on semi-permeable membranes separating the top culture chamber from a bottom microfluidic channel. Endothelial cultures can be established either with single cell type as monocultures, or with tissue specific epithelial cells or organoids grown on the top culture chamber for more fine-tuned and complex models. The structure of AKITA® Plate allows ease-of-use induction of flow with a rocker, compatible with standard cell culture equipment reducing the set-up costs. Barrier formation can be visualized on the plate with live/dead staining and assayed by measuring electrical resistance or the permeability of fluorescent probes. As a proof of concept, we have established endothelialized tissue models such as the lung-on-chip, blood-brain-barrier-on-chip, and gut-on-chip.

RESULTS: Cultured under dynamic conditions, our endothelialized tissue models showed increased barrier tightness. For tissue specific co-cultures it has been noticed that the addition of endothelium increases the barrier properties both in permeation assays as well as for functional performance in studies such as inflammation tests.

DISCUSSION & CONCLUSIONS: We introduce a microfluidic high throughput commercially available platform for the modelling of vascularized organ interfaces. Compatible with standard 96- and 384-well plate formats, our AKITA® Plates are easy to handle and compatible with automatized confocal imaging processes. Tissue models grown on AKITA® Plate can be used for compound permeation studies or personalised medicine screening in a patient-relevant manner. Additionally, our standardized and translational platform offers an alternative for animal testing, reducing the costs and time that is now used in preclinical drug testing.

Keywords: Vascular systems / vascularisation and heart, Scaling up & manufacturing

PP-382

3D microphysiological placenta in-vitro model as a tool for drug transport studies and risk assessment

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INTRODUCTION: The human placenta is a unique organ that acts as the interface between the mother and the fetus. Its multilayered structure that consists of the syncytium and the fetal endothelium, separated by a thin connective tissue, is essential for the exchange of various substances and to protect the fetus from xenobiotics. Considering that over the past decades the intakes of medication during pregnancy are on rise, risk assessment of drug therapies during pregnancy is gaining more importance. Unfortunately, the current widely used in vitro translocation models do not fully resemble the physiological structure and fail clinical translation. Here we present a in vitro model of the placental barrier toward the assessment of drug transport using the microfluidics OrganoPlate® 3-lane. This platform is based on a 384-microtiter plate which allows for a parallel culture of 40 perfused miniaturized placenta barrier, in a membrane-free manner.

METHODS: To model the placenta barrier on-a-chip, the trophoblast cell line BeWo b30 and primary human vascular endothelial cells HUVEC were grown against a thin connective tissue-like extracellular matrix (ECM) in the OrganoPlate®. The ECM can be freely patterned in microchannels using PhaseGuide™ technology (capillary pressure barriers). Perfusion through the lumen of maternal epithelium and fetal endothelium is induced without pump and can be controlled to model mechanical cues.

RESULTS: Syncytium formation, polarization and specific marker expression were characterized at the protein and mRNA level. Upon perfusion flow, trophoblast and endothelial cells formed lumenized, leak-tight confluent tubular structures. Barrier tissue integrity was confirmed through TEER (transepithelial electrical resistance) measurements. Syncytium formation was characterized by a break-down of tight junction (ZO-1), and an increase in hCG-β expression. The activity of major placenta barrier drug transporters, such as multidrug-resistance protein (MRP), was evaluated through in- and efflux transporter assays. Finally, drug-induced toxicity was assessed by syncytium apical exposure to common drugs taken during pregnancy, known substrates of MRP. Cellular damage was assessed, and barrier disruption was evaluated by TEER measurements using the OrganoTEER®, a device fully compatible with the OrganoPlate®. Exposure of the maternal compartment to compounds showed dose-dependent cellular damage and barrier integrity loss.

DISCUSSION & CONCLUSIONS: The placental barrier on-a-chip provides a complex co-culture model to recreate an in vivo-like microenvironment. Suitable for transport studies and high-throughput drug-induced toxicity screening, this model provides valuable insight for drug risk assessment during pregnancy.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Developmental biology

PP-383

Vascular inflammation modulates Trans-Endothelial Electrical Resistance and immune cell migration in a scalable organ-on-a-chip platform

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INTRODUCTION: The vasculature system plays a critical role in the body inflammation processes. Vascular inflammatory mechanisms are characterized by disruption of blood vessel wall permeability together with increased immune cell recruitment and migration. There is a critical need to develop models that fully recapitulate changes in vascular barrier permeability in response to inflammatory conditions.

METHODS: To this extent, we demonstrate a platform for inducing inflammatory reaction in HUVEC based microfluidic barrier models combined with continuous Trans Epithelial Electrical Measurements and coculture with Human peripheral Blood mononuclear cells.

RESULTS: We found that the endothelial barrier changes differently depending on the inflammatory cytokine or immune cells added leading to changes in ICAM expression and endothelial morphology.

DISCUSSION & CONCLUSIONS: We found that the endothelial barrier changes differently depending on the inflammatory cytokine or immune cells added leading to changes in ICAM expression and endothelial morphology. We propose our platform as an essential tool for modeling endothelial inflammation in combination with immune cell interaction that can be used to screen for different targets and drugs to treat chronic vascular inflammation.

Keywords: Immunity / immunomodulation / macrophage, Vascular systems / vascularisation and heart

PP-384

A human organoid-derived cholangiocyte model in a perfusable microfluidic platform

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INTRODUCTION: Challenges in attaining adult-like maturation of liver epithelial cells, lead researchers to look beyond differentiating cells from pluripotent stem cells or cell lines of tumor origin. The establishment of liver organoids from isolated adult hepatic epithelial cells is currently at the innovative forefront of in vitro liver modeling. Intra-hepatic cholangiocyte organoids, resulting from liver progenitor cells of biliary identity, have emerged as a powerful tool to study cholangiocyte biology, as well as providing an expandable cell source. These organoids typically consist in extracellular matrix-embedded cyst-like monolayers enclosing a hollow apical lumen. Solving the intrinsic heterogeneity of organoid cultures as well as the inaccessibility of the apical lumen are vital next steps for full adoption of organoid technology in the industry and clinics. Microfluidics offer the possibility to carefully pattern the cells in a confined space.

METHODS: Here we coupled organoid technology and microfluidics to establish a standardized, fully accessible and perfusable human cholangiocyte organoid model from three different donors. After growing as traditional hydrogel-embedded organoid cultures, the cells were extracted and seeded inside a microfluidic channel adjacent to a collagen-I interface.

RESULTS: We observed rapid cell attachment, as well as proliferation, leading to the formation of complete tubular structures. These tubes presented an intact and stable barrier function, active and directional transport as well as mature cholangiocyte phenotype. Basolateral and apical accessibility of the system allowed us to challenge the cholangiocytes with a range of bile acids while simultaneously monitor the integrity of the barrier. Depending on the hydrophobicity and the administered location of the bile acids, we found profound differences in their biological effects, ranging from high toxicity and barrier disruption to increase of barrier function.

DISCUSSION & CONCLUSIONS: In conclusion, we present a standardized, reproducible, mature and perfusable cholangiocyte organoid model that recapitulates the three-dimensional structure as well as the function of the human bile duct.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Stem cells - general

PP-385

Optimization of synthetic hydrogels for the manufacture of 3D organoid model for drug discovery

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INTRODUCTION: Monolayer cell culture is a crucial tool used in drug discovery, immunology, and many other research fields. Although this method is widely used it lacks in-vivo features such as spatial organization, cell-to-cell interaction, and cell polarization amongst others. Due to this deficiency, developmental biology and cell biology fields lead to the discovery of 3D cell cultures, a more accurate method that implements the in-vivo features is needed. Organoid growth is one area that is attracting significant attention, because organoids are in-vitro three-dimensional clusters of cells that self-organise and reassemble organ functions. This method typically uses the pluripotency of stem cells and subsequent tissue differentiation is controlled by an intrinsic process called organogenesis. To sustain the organoid formation this cell culture typically needs a scaffold to support growth and these come from either natural or synthetic sources. The most common scaffold used in research is Matrigel, an animal-derived mixture of ECM proteins originated from tumour mice Englebert-Holm-Swarm cells. This has obvious ethical issues, and the known batch-to-batch variability leads to a lack of reliability and reproducibility in end applications, including for example in drug discovery which can lead to a loss of resources, loss of time and sometimes the wrong clinical result carried through to clinical trial.

METHODS: 3D Cell encapsulation, Oscillatory rheology, Albumin/urea assay

RESULTS: Here we will report on cell viability and organoid growth for a range of self-assembling peptide hydrogels and identify the optimal hydrogel for liver organoid growth. More specifically, we will show that running the cell culture without Serum has minimal impact on cell viability, while results of cell culture within a range of different stiffnesses and different charged peptide hydrogels will be presented paying particular attention to organoid growth rate, number, size and functionality. Final studies comparing the cell metabolism will be shown highlighting the potential to swap animal derived Matrigel with fully synthetic peptide hydrogels.

DISCUSSION & CONCLUSIONS: This project explores the potential of fully synthetic peptide hydrogels as a replacement for animal-derived Matrigel for organoid growth and culture. We will introduce a deep understanding of the self-assembly peptide hydrogel and the importance of its physical, mechanical, and biological properties in organoid growth. We will go onto give an overview of the development of different organoid tissues and how their differentiation is guided, before concluding with highlighting the potential of organoids in drug discovery and address the challenges that are still to be solved.

Keywords: Hydrogels and injectable systems, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-386

TTOP static preparation system: a versatile and scalable MicroPhysiological device for compartmentalized and easily accessible cultures of tissue barriers

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INTRODUCTION: Micro Physiological Systems (MPSS) technology is a valuable tool when replicating in vitro tissue barriers, showing more predictive results than conventional in vitro or animal models. However, MPSS are still considered not mature enough and their penetration in the pharmaceutical industry is still limited by manufacturing and industrial hurdles [1]. For these reasons we developed True Tissue On Platform (TTOP), a scalable and versatile platform, that aims at replicating tissue barrier functions, enabling a fast integration in laboratory procedures.

METHODS: The platform was designed to enable cleanroom-free and PDMS-free fabrication. A 350 micron, laser micromachined cartridge was positioned in an open-well static module compatible with standard 12-well plates, enabling conventional handling requirements. Thanks to the open-well design, we have been able to host microporous membranes, on which culturing Caco-2 cells, as a model of the intestinal epithelium, or human reconstructed 3D intestinal tissues (MatTek EpiIntestinal™[2]). To demonstrate the functionality of the device, we monitored the cell/tissue cultures by both TEER measurements and Hoechst living staining evaluating tissue barrier functions. Finally, we retrieved the cartridge with a simple procedure and performed confocal imaging and TEM to verify the expression of membrane and junctional markers (HEA, JAM-A, ZO-1 and Villin).

RESULTS: The fabrication procedures allowed to obtain up to 70 devices (static module + cartridge) with an easy-to-replicate protocol in a cleanroom-free environment. Caco-2 growth was monitored every 2 days, showing an increase in TEER values in accordance with cell distribution, observed by light and fluorescent microscopy. At the end of the culture, the cartridges were easily retrieved and fluorescence microscopy showed that Caco-2 were able to form mature and polarized monolayers after 21 days. Similarly, EpiIntestinal™ 3D intestinal tissues demonstrated comparable TEER values in TTOP vs. control. Moreover, confocal microscopy and TEM confirmed the presence of the brush border, tight junctions, and allowed to investigate the microtissue 3D morphology.

DISCUSSION & CONCLUSIONS: TTOP shows to be a promising new technology, enabling accessible and cost-effective devices for barrier tissues in-vitro replication, allowing a fast integration in laboratory procedures. Moreover, the versatility of the platform allowed to host microporous membranes as well as 3D organotypic tissues enabling studies from simpler to more complex in vitro models. Finally, the cartridge-based controlled retrieval allowed to perform confocal imaging and TEM without affecting the morphological structure and integrity of the biological sample.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In vitro microenvironments

PP-387

A 3D *in vitro* model of breast cancer model using decellularized mammary glands bioinks

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INTRODUCTION: Breast cancer is the most common cancer in women. Despite the great deal of resources invested to discover new treatments, only a small percentage of these ever reach patients, mainly because current preclinical models are not fully translatable to clinical trials. 3D bioprinting can assist in preclinical testing by providing models that recreate the architecture and physiology of the tumor microenvironment (TME),¹ which is formed by breast cancer cells (BCCs), stromal cells and extracellular matrix (ECM). In addition, the importance of the ECM in tumor progression and drug resistances, has motivated the development of bioinks that recapitulate the ECM complexity. In this regard, decellularized tissues-derived matrices (TDMs) can provide the native tissue biological cues, but its inadequate mechanical properties prevent their bioprinting. The aim of this work is to develop a 3D *in vitro* breast cancer model using breast TDM-derived bioinks.

METHODS: TDM bioinks were prepared by mixing gelatin-methacrylamide (GelMA), alginate and decellularized and enzymatically digested porcine mammary glands.² Bioinks were further tuned by incorporating Collagen 1 (Col1), an ECM protein overexpressed in breast cancer. TDM bioinks were printed with a 3D bioplotter and crosslinked. Bioinks' shape fidelity, printability and mechanical properties were characterized. For bioprinting artificial tumors, breast cancer cells (BCCs) or mesenchymal stem cells (MSCs) were dispersed in the bioinks. Cellular survival, proliferation, morphology, invasion, expression of adhesion molecules, malignancy and drug resistance markers and drug efficacy were studied. Col1 gels were used as controls.

RESULTS: The alginate and GelMA addition to breast TDM enable its bioprinting with appropriate printability in the absence of a sacrificial material and recreating tumor stiffness. BCCs were able to proliferate in TDM bioprinted scaffolds, forming spheroids and cell networks. BCCs showed lower expression of e-cadherin, higher expression of fibronectin and multidrug resistance protein-1 and higher resistance to doxorubicin than controls. BCCs showed a matrix metalloproteinase-dependent invasion in TDM scaffolds. 3D models of MSCs and BCCs could be successfully bioprinted with TDM bioinks.

DISCUSSION & CONCLUSIONS: Taken together, we have shown that TDM bioinks could be used to bioprint breast cancer models, closely recreating cancer cell malignancy and tumor ECM.

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Keywords: In vitro microenvironments, 3D printing and bioprinting

PP-388

An in vitro model system of intracerebral haemorrhage

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INTRODUCTION: Despite the global health burden, intracerebral haemorrhage (ICH) is a devastating neurovascular attack with limited treatment options. Although mammalian models of ICH have improved our understanding of disease mechanisms, pre-clinical drug trials have thus far failed in terms of clinical translation. Alternative, complementary pre-clinical approaches are required to identify and trial drug compounds.

METHODS: In this study we have used alginate hydrogel to mimic the 3D structure of the haematoma and damaged tissue that is observed physiologically. Alginate is able to form hydrogel easily with the addition of divalent cations, here Ca²⁺, upon which α -L-guluronic acid monomers are bound into 'egg box' structure¹. Whole blood was mixed with alginate (0.5% w/v) and encapsulated into ~150-250 μ m hydrogel beads in 0.5M CaCl₂. Beads were then encapsulated in a second layer of alginate (0.5% w/v) containing hyaluronic acid/chitosan nanoparticles to model the microenvironment of damaged brain tissue surrounding the haematoma.

RESULTS: Physical properties of the model haemorrhages were characterised including size, material rheology and the release of fibrinolysis factors over time. Model haemorrhages were then cultured with human monocytes and neuronal cell lines and the resulting cellular survival and migration and inflammatory profile was characterised.

DISCUSSION & CONCLUSIONS: In conclusion, we have started to develop an in vitro model of ICH with the potential to be widely adopted to aid with pre-clinical modelling, drug discovery and development in ICH research, with a view to replace protected species currently required for disease modelling.

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Keywords: Nervous system (brain-central-peripheral / disorders), Cardiovascular

PP-389

In vitro model for intervertebral disc degeneration

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INTRODUCTION: Low back pain (LBP) causes disability and life quality deterioration, constituting a tremendous social and economic burden. 60-80% of adults experience varying degrees of low back pain. In more than 40% of the cases, it is triggered by intervertebral disc (IVD) degeneration. Conventional therapies for LBP predominantly involve treatments based on pain modulators and invasive surgeries, like spine fusion or arthroplasty. However, spine surgeries have a high risk of complications and recurrent interventions are needed. Although rapid advances are being made in understanding and regulating the degenerative process, many challenges remain. Understanding IVD pathophysiology (particularly in terms of IVD matrix constituents and their alterations in development and disease) is key to unveiling molecular cues that might be used to slow, halt or reverse the degenerative cascade.

METHODS: In this work, we aimed to develop an in vitro intervertebral disc degeneration (IVD) model to answer important questions regarding the main factors involved in disc degeneration. Such a model has the potential to unveil biochemical, cellular and biomechanical pathways of this pathology. The model employs tissue-specific cells, biomaterials and culture conditions that mimic the cellular environment of the degenerated human intervertebral disc. To this end, three dimensional IVD constructs were developed with varying concentration of collagen I and proteoglycans (PGs) mimicking nucleus pulposus (NP) tissue (the core gelatinous cartilage-like tissue in the intervertebral disc) and collagen II, proteoglycans mimicking anulus fibrosus (AF) (the layer surrounds NP). Human NP and AF cells were encapsulated in the collagen-PGs gels. Cell morphology, the expression of specific markers including collagen type I, collagen type II and the PGs secreted by the cells, cellular regulators such as hypoxia inducible factor (HIF1- α), matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were assessed.

RESULTS: Higher expression of HIF-1 α and higher secretion of PGs by NP cells observed as collagen concentration increased in the constructs. This result shows that hypoxia increased the secretion of PGs mostly by NP cells. However, there was no significant differences in the PGs quantities secreted by AF cells in the constructs. At high concentrations of collagen, metalloproteinase-1 (MMP-1) significantly increased in the AF cells whereas tissue inhibitor of metalloproteinase-1 (TIMP-1) decreased in all NP and AF in all constructs.

DISCUSSION & CONCLUSIONS: IVD constructs showed capabilities to regulate cellular markers while controlling HIF1- α activation within cells.

Based on our results, the reduction in collagen and proteoglycan contents of the intervertebral disc play a significant role in tissue degeneration process.

Keywords: Biomaterials, Hydrogels and injectable systems

PP-390

Toward 3D cellular bioprinting of age-related macula degeneration-in-a-dish

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INTRODUCTION: Age-related macula degeneration (AMD) is a blinding neurodegenerative disease of the retina. It affects 4% of the population over 60. For over 90% of patients, there is no cure due to cellular atrophy of the retina. The outer blood-retina barrier is the affected tissue in AMD. The disease starts with a progressive accumulation of deposits underneath the retina, accompanied by local stress, new vessel formation, and immune response. The development of new AMD therapies is essentially hampered by the lack of a representative model. Our aim is to establish a controllable, vascularized in vitro human 3D model for AMD by bioprinting endothelial cells basally on an artificial Bruch's membrane of the retina. The model will be compatible to be combined with other relevant retina cells.

METHODS: A human umbilical vein endothelial cells (HUVEC) cell line was used for pilot experiments, which is a well-established model system for studying endothelial cell function. Stem cell-derived endothelial cells were also generated, using a protocol, adapted from a stepwise differentiation protocol for stem cell-derived choroidal endothelial cells. Alginate- or peptide-based hydrogels were tested for printability (5% Alginate, Alginate-RGD, Alginate-Fibrin, IKVAV peptide, Alginate-RGD-Fibrin, IKVAV peptide-Alginate-Fibrin). HUVEC cells were embedded in the hydrogels, cultured for 7 days. The 3D model was printed using a RegenHU 3D Discovery bioprinter. The model was tested for printability and endothelial cells were characterised for morphology, for endothelial cell-specific markers using light microscopy, confocal scanning microscopy, for network formation using conventional tube formation assay and for toxicity measuring lactate dehydrogenase release. Images were analysed using ImageJ.

RESULTS: The embryonic stem cell differentiated endothelial cells were positive for endothelial cell-specific CD31 and CA4 and stored for further development of the bioprint model. In parallel, alginate- or peptide-based hydrogels showed stable line printing ability, with up to 3 layers to be printed using peptide-based hydrogel within a diameter of 1.2 mm surface area. HUVEC cells showed better cell spreading and network formation ability in hydrogels, when fibrin was added (number of nuclei 35 and 37 without fibrin and 57 and 48 with fibrin, respectively). HUVEC cells, which were embedded alginate- or peptide-based hydrogels, showed less relative cell toxicity with the addition of fibrin, than without (1.4 ± 4 and 3.3% with fibrin and 4.7 ± 4 and 5.7 ± 2 without fibrin, respectively).

DISCUSSION & CONCLUSIONS: In summary, a retina model for choroidal vasculature was established and fibrin appears to be essential for proper endothelial cell behaviour, as a first step toward printing AMD-in-a-dish.

Keywords: Eye,

PP-391

Taurine Supplementation against Steroid Myopathy in 3-D in vitro Skeletal Muscle Tissues

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INTRODUCTION: Skeletal muscle mass wasting or atrophy is a common observation in multiple diseases due to increased levels of glucocorticoids or synthetic glucocorticoid-based anti-inflammatory therapies. It is, therefore, pivotal to identify and validate potential therapeutic approaches to counteract glucocorticoid-induced muscle atrophy. This research employed a 3-D in vitro human skeletal muscle platform to model structural and functional phenotypes associated with Dexamethasone-induced muscular atrophy.

METHODS: The model comprised of Matrigel-Fibrinogen encapsulated human satellite cells on a PDMS platform with flexible pillars to provide requisite tensile force enabling muscle maturation. The contractile force was evaluated using Electric Pulse Stimulation with square pulses of 10V and varying frequencies (1Hz, 2Hz, 5 Hz, 10 Hz, 25Hz and 50Hz) to produce both twitch and tetanic contractions. The glucocorticoid atrophy model was developed by challenging the tissues with Dexamethasone for; a) 24 hours and b) 48 hours. Taurine rescue was introduced for 24 hours: a) after 24 hours of Dexamethasone treatment and b) in combination with dexamethasone (48 hours).

RESULTS: Tissues treated with Dexamethasone (100uM) indicated a decline in contractile force and a significant reduction in myotube diameter. Functional recovery of contractile force post Taurine treatment was observed as well as a significant gain in myotube diameter. Atroge levels were also studied after rescue with Taurine to further validate atrophy and atrophic recovery.

DISCUSSION & CONCLUSIONS: Skeletal muscles are the largest and most adaptable protein reservoirs with a capacity to regulate their mass and fiber cross-sectional area in response to a variety of external stimuli. Endogenous glucocorticoids are among the first known energy regulators of the human body[1]. They function by metabolizing carbohydrates, fats and proteins. As a reservoir of amino acids, glucocorticoids act on skeletal muscles to increase free serum aminoacid, resulting in a significant loss of muscle mass [2]. First discovered in the Cushing's disease, glucocorticoid-mediated muscular atrophy has since been commonly observed in patients receiving glucocorticoid therapies. Contemporary pathophysiological research utilizes samples from patient biopsies or rodents to simulate and study these pathways, which often have limited reproducibility in humans. 3-D in vitro tissues bioengineered from patient-derived cells have proved to be a suitable platform to not only procure patient-specific pharmacologic responses but also to curb monetary bleeding.

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Keywords: Disease models, Musculoskeletal (inc ligament / tendon / muscle / etc)

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Villains and victims – Deciphering the impact of healthy and diseased cell-types on cardiac fibrosis using hiPSC derived cardiovascular models

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INTRODUCTION: Heart attack leads to fibrotic scar tissue formation known as cardiac fibrosis, which leads to heart failure with 50% 5 year mortality rate. There is a need for better cardiac fibrosis models for patients with chronic heart failure and underlying heart disease¹. We evaluated isogenic genome-edited human induced pluripotent stem cells (hiPSC) harbouring p.R453C- β -myosin heavy chain (β -MHC) mutation underlying inherited condition, hypertrophic cardiomyopathy (HCM)².

METHODS: The isogenic set of hiPSC- β MHC-R453C were differentiated into cardiomyocytes (hiPSC-CMs), cardiac fibroblasts (hiPSC-CFs) and endothelial cells (hiPSC-ECs) using our customised serum-free culture protocols. Effects of surface stiffness on 2D standard tissue culture plastic plates (3GPa) and modulated substrates (25kPa and 2kPa) in myofibroblasts (hiPSC-MFs) activation was investigated. Function of hiPSC-CMs, hiPSC-ECs and hiPSC-CFs was assessed by contractility, angiogenesis and hiPSC-MFs activation respectively. 3D tri-lineage culture as spheroids, encapsulation in defined peptide gels or biofabrication printing techniques are currently in development.

RESULTS: Animal-free protocols for high-purity (>85%), reproducible differentiations of hiPSC-CMs, hiPSC-ECs and hiPSC-CFs have been developed by replacing animal serum products. Substrate properties and culture conditions were optimised for maintaining hiPSC-CF phenotype and preventing conversion into unwanted hiPSC-MFs. We fine-tuned TGF β modulation and identified need for further substrate optimisation from the standard tissue culture plastic plates. Significant changes between isogenic healthy and diseased lines in contraction and fibrotic responding genes provide an exciting new toolkit for disease modelling. Co-cultures of hiPSC-CMs with hiPSC-CFs on polymer functionalised micropatterned substrates, which are tunable for stiffness are being investigated to better recapitulate fibrotic processes.

DISCUSSION & CONCLUSIONS: Characterisation of healthy and diseased lines and optimisation of serum-free protocols enable us to take the next steps in using complex model systems including biofabrication to control architecture of hiPSC-CMs, hiPSC-CFs and hiPSC-EC tri-cultures and understanding the mechanisms of cardiac fibrosis. Our property genetically modified diseased lines provide a powerful tool for us to simulate different disease severities. This will allow us to isolate and identify main disease regulators ("villians") among the different cell lineages ("victims") being investigated highlighting the importance of physiologically and genetically relevant models. The validation of the humanised models with serum-free conditions will also improve translational applications by removing the need for animal use.

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Towards modeling the joint on a chip: a mechanically active microfluidic system to engineer 3D multi-layer osteochondral tissues

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INTRODUCTION: Osteoarthritis (OA), the most prevalent musculoskeletal disease, is a degenerative disorder mainly affecting load bearing joints. Correlated with mechanical dysregulation, OA leads to cartilage degeneration but also to its vascular invasion, and to subchondral bone alterations¹. No disease modifying OA treatment is presently available, also due to the absence of satisfactory comprehensive models incorporating the subchondral layers. In this work we propose a new Organ-on-Chip (OoC) concept that allows for the easy generation of stacked, directly interfaced 3D multi tissues microconstructs. Leveraging on this technology we pioneer a new mechanically active joint OoC comprehensive of both a cartilage and a (vascular)subchondral compartment.

METHODS: Building upon our Cartilage on chip model² we engineered, through multi-layer photolithography, a mechanically active device with a valve system to pin a suspended fluid so that two subsequent injections can be realized, generating a multi-layer construct. A co-culture joint model was realized with chondrocytes (CHs) for the cartilage layer and a combination of mesenchymal stromal cells (MSCs) and HUVECs for the mineralized vascular compartment. GFP expressing CHs were used to evaluate the effect of the compression in the different layers through RT-qPCR. Cells were embedded in an enzymatically formed, metalloproteinase (MMP) sensitive PEG hydrogel.³

RESULTS: The device was validated demonstrating production feasibility and functioning of the vertical valve concept. Computational simulations adopted to estimate the strain field revealed a strain gradient (ranging from 30% to 0.3%) reminiscent of that of in vivo OA joints.

Immunofluorescence images of the co-culture demonstrated the possibility of achieving positionally defined complex tissues with a cartilaginous layer interfaced with a vascular zone, towards a complex osteochondral-like unit. The distinct compression levels applied to cartilaginous microconstructs hosted in the two device compartments induced differences in the expression of mechanotransduction related genes 4 (PTGS2, C-JUN) but also in that of OA markers such as degradation related genes (MMP13), and release of the inflammatory cytokine IL8.

DISCUSSION & CONCLUSIONS: The introduction of this new vertical, fluid pinning OoC concept paves the way for more representative OoC joint models where phenomena such as cartilage degradation and vascular invasion can be dissected in vitro and innovative OA drug targets and regenerative therapies tested.

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Keywords: Cartilage / joint and arthritic conditions, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

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Replication of electrospun fiber textures in different polymers for cell-matrix interaction studies

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INTRODUCTION: Electrospinning is one of the key technologies to fabricate nano- and microscale fiber meshes with tunable fiber diameter and material properties to mimic specific physical cues of the cell microenvironment.¹ However, it is intrinsically challenging to produce not only similar but identical fiber textures multiple times and even in different materials via conventional electrospinning, which is crucial to reproduce and evaluate the impact of surface properties and materials on cell behavior. To overcome this limitation, we propose a novel multi-replication process of electrospun fiber textures.

METHODS: We used electrospun fiber mesh as a master template and replicated fiber topographies onto commercially available biocompatible polymer films, such as polycarbonate (PC), polystyrene (PS), poly(methyl methacrylate) (PMMA), and cyclic olefin copolymer (COC) by nanoimprint lithography (NIL). The morphology of electrospun fibers, moulds and fiber imprints were characterized using scanning electron microscopy. To demonstrate the use of our fiber texture replication technique, we assessed the morphology and alignment of human umbilical vein endothelial cells (HUVECs) on the different imprinted films.

RESULTS: The characterization of the fiber architecture and fidelity of the multi-replication process showed that spatial distribution and randomness of fiber topographies is well preserved between the electrospun mesh and the polymer imprints. The average fiber diameters of random and aligned electrospun fiber meshes varied between 0.3 – 2.8 μm , which was also measured on imprinted polymer films. In our cell culture studies, the measurements of cell shape orientation showed a preference for alignment with the replicated fiber patterns, whereas on randomly oriented fiber imprints and smooth PC films, HUVEC's showed a random angle distribution and thus no preference in orientation.

DISCUSSION & CONCLUSIONS: Our innovative process enables the faithful replication of textures of electrospun fiber meshes and for the first time a complete decoupling of fiber textures and material properties. The replicated fiber texture templates can successfully be transferred to various biocompatible polymers, which can be used in high-throughput cell culture studies. In the future, our technique will pave the way to identify and elucidate more subtle cell responses to complex, extracellular matrix-like fiber textures through the possibility to increase sample sizes enabling a more robust statistical analysis.

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Deciphering the Role of Hedgehog Signalling in the Osteoinductive Influence of Topographically-Textured Polymeric Microparticles

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INTRODUCTION: The osteogenic commitment of mesenchymal stromal cells (MSCs) is regulated by biochemical and mechanical cues within their niche. Hedgehog signalling has a well-known role in bone development and osteogenesis by upregulating the expression of osteogenesis-associated genes through activation of the Gli family of transcription factors, of which Gli1 (Glioma-associated oncogene homolog 1) is both a mediator and a marker of pathway activation. Gli transcription factors play key roles in mesodermal tissue patterning and osteogenic differentiation in response to external stimulation. Tailoring three-dimensional (3D) substrate architectural features induces osteogenic response in MSCs *in vitro* [1]. However, the molecular mechanisms of this osteoinduction have not been determined.

METHODS: To evaluate the role of the Hedgehog-Gli1 pathway in this osteoinductive response to 3D topographically-textured biomaterials, murine embryonic mesoderm fibroblast C3H10T1/2 cells were utilised as a model system for MSCs, because of their multilineage differentiation potential and functional similarity to bone marrow derived MSCs. Cells were cultured on smooth and dimpled poly(DL-lactide) (PDLLA) microparticles to assess differences in viability, proliferation, cellular morphology and the expression of Hedgehog signalling- and osteogenesis-related genes. Relative quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression levels of Patched 1 (*Ptch1*), *Gli1*, and Runt-related transcription factor 2 (*Runx2*) at different time-points. Cells were treated with the Smoothed (Smo) antagonist KAAD-cyclopamine, a Hedgehog signalling inhibitor, to evaluate the involvement of Smo in Gli target gene activation, and data was normalised to untreated controls.

RESULTS: Cells proliferated on microparticles, assessed for up to day 10, and displayed high viability under low serum conditions. Cell morphology was influenced by microparticles topographies, with cells spreading on smooth microparticles and showing more elongated morphologies on dimpled microparticles. Activation of the Hedgehog pathway was observed in the cells cultured on dimpled microparticles, displaying higher gene expression levels of *Ptch1* and *Runx2*. *Gli1* expression was significantly upregulated in dimpled compared to smooth microparticles ($P < 0.0001$), with a 19-fold change relative to 2D controls. Moreover, *Gli1* induction in dimpled microparticles was prevented by treatment with KAAD-cyclopamine.

DISCUSSION & CONCLUSIONS: This study suggests that the canonical Hedgehog signalling may mediate the osteoinductive effect of 3D topographically-textured microparticles on C3H10T1/2 differentiation. Further characterisation of this model system will advance our understanding of the

molecular mechanisms underlying cellular mechanoresponsiveness to engineered biomaterial topographical features. These findings will inform bone tissue engineering strategies and allow modulation of Hedgehog signalling *in vitro* for high-throughput drug screening applications.

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Keywords: Biomaterials, Stem cell niche

PP-396

Mimicking the physiology of multiple layers of human subcutaneous adipose tissue using adipose stromal/stem cells spheroids from superficial and deep subpopulations

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INTRODUCTION: Adipose tissue engineering may provide 3D constructs for clinical transplants as well as disease models such as obesity. In human subcutaneous adipose tissue (SAT), the superficial layer has been described as more hyperplastic and angiogenic while the deep layer as having pro-inflammatory capacity. Recently, our research group described adipose stem/stromal cells (ASCs) subpopulations derived from multiple layers of human SAT (Baptista et al., 2021). In this study, spheroids were generated from ASCs derived from superficial (sSAT) and deep (dSAT).

METHODS: ASC spheroids were formed in micro-molded nonadhesive hydrogels with 81 circular recesses fabricated according to manufacturer's recommendations (MicroTissues 3D Petri Dish) and maintained under adipogenic induction media (Baptista et al., 2021) for up to 5 weeks in the presence or absence of a pro-inflammatory stimulus with lipopolysaccharide (LPS). The adipogenic potential was evaluated by positive staining of intracytoplasmic lipids droplets (Nile Red O staining) and by Real Time PCR for the mRNA expression levels of FABP4 and CEBP α genes. The pro-inflammatory capacity was evaluated by a lipolysis assay (colorimetric assay kit) and by the dosage of cytokines and growth factors in the culture supernatant (multiplex assay).

RESULTS: ASC spheroids from sSAT revealed the highest adipogenic potential supported by unilocular lipid droplets and the highest levels of FABP4 and C/EBP α mRNA expression and from dSAT the highest lipolytic capacity, as expected. Curiously, ASC spheroids from dSAT did not show the highest levels of proinflammatory secreted molecules, even in the presence of LPS. ASC spheroids from sSAT showed a higher synthesis of VEGF compared with dSAT ($p < 0.0001$).

DISCUSSION & CONCLUSIONS: This study opens the possibility for an optimized adipose tissue engineering, since distinct layers can be formed in vitro according to specific needs.

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Keywords: Multipotent (mesenchymal) stem cells, Stem cell niche

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Cost-effective 3D lung tissue as a model for SARS-CoV2 infection and drug testing

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INTRODUCTION: The Severe acute respiratory coronavirus 2 (SARS-CoV-2) primarily infects lung cells. Due to COVID-19 pandemic, the development of lung organoids was needed, increasing the number of scientific publications in this field. However, organoid faces critical challenges such as high costs and reproducibility not representing the best choice for high throughput assays for drug testing (Kronemberger et al., 2021). In this study, we developed a cost-effective and reproducible 3D lung tissue model consisting of human lung epithelial and stromal cells in a spheroid model (submitted patent).

METHODS: Spheroids were inoculated with one multiplicity of infection (MOI) of the RJ1 variant of SARS-CoV2. After 2 hours of incubation, the medium was renewed and the culture was maintained at 37°C and 5% CO₂ for up to 48 hours. Spheroids were fixed and processed for histological and immunohistochemistry analysis and for transmission electron microscopy (TEM) at 24 and 48 hours post infection (hpi). The spheroid culture supernatant was collected to cytokines dosage (multiplex assay).

RESULTS: Morphologic analyses review a decrease of stromal diameter in 24 and 48 hpi (25,14% and 29,68% respectively) and an increase of epithelial layer diameter in 24 and 48 hpi (18,84% and 31,88% respectively). Histologic analysis shows cell death in both layers, despite epithelial cells being suggested to be more susceptible. At 24 and 48 hpi, electron microscopy analysis exhibits the presence of Large virion-containing vacuole (LVCV) and genetic material in double-membrane vesicles (DMV). Immunohistochemistry for viral proteins and cytokine dosage analysis are in progress.

DISCUSSION & CONCLUSIONS: The present 3D lung tissue model has been validated for SARS-CoV2 infection assays. Other variants are being tested and comparisons are being established related to their respective degrees of infectivity.

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Keywords: Infection, Disease models

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Cell-Gym On Chip: A Microfluidic Platform For Cell Wellness Control

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INTRODUCTION: Healthcare research focuses on the study of new strategies to extend the human lifespan. The in vitro control of cell behavior, commonly mediated by genetic approaches, can be complemented by the epigenetic manipulation, which focuses on the influence of external factors on the cell microenvironment composition. Many catabolic processes physiologically improve cell health when detrimental signals are applied. It is then conceivable that cell wellness could be enhanced by exploiting these phenomena. The healthy state can be improved in terms of autophagy, which is a cell catabolic process that preserve it from detrimental extracellular signals by degrading or recycling worthless metabolites in vesicles [1]. All these concepts were applied in this work to design a cell training platform that reveal the conditioning program able to reproduce a healthy cell state by applying a combination of signals through a microfluidic platform.

METHODS: In the microfluidic platform parallel conditions of biochemical concentrations were tested in a gradient logic and each chamber was enriched with a stretchable membrane for mechanical stimulation unit. Here, HL-1 cardiomyocytes survival and response to microenvironment manipulation was checked by cytoplasmic staining. Autophagy in HL-1 cells was tracked by transfecting them with a fluorescent reporter that tagged to autophagic vesicles to control autophagy enhancement following the epigenetic stimuli.

RESULTS: Epigenetic manipulation due to starvation and equibiaxial stretch was applied in a microfluidic context for highly controllable environment and was used to induce autophagy in cardiomyocytes cell line. In particular, autophagy was enhanced by these external stressors with respect to steady-state cell culture conditions, along with low percentage of cell death. Tests on the microfluidics demonstrated that fluid flow did not impair the deformability of the membrane. The microfluidic apparatus supported HL-1 cells survival and proliferation, the mechanical stimulus conditioned cell orientation and migration in time, while cell survival changed following starvation induction.

DISCUSSION & CONCLUSIONS: This work demonstrated how it is possible to combine microenvironment signals within the same platform by applying microfluidic approaches to cell culture. Cardiomyocytes autophagy enhancement is the pathway chosen by the cells to recover their health under the conditioning of microenvironment signals, so that it is possible to quantify cell health with a direct correspondence between stimuli level and autophagy state.

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Keywords: Interfaces - engineered, Biomechanics / biophysical stimuli and mechanotransduction

PP-399

The Influence of the Degenerating Nucleus Pulposus Microenvironment on Cellular Matrix Synthesis Rates

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INTRODUCTION: The microenvironment of the intervertebral disc (IVD) is heavily reliant on diffusion of nutrients and metabolites through the cartilage end plate (CEP) and therefore varies with increasing levels of degeneration. Degeneration of this microenvironment can impact the outcomes of regenerative therapies as it heavily influences the function and survival of the intradiscal cells. Key microenvironmental parameters within the IVD have been previously characterised [1,2] but their effect on the cellular response has yet to be fully elucidated. The aim of this work is to determine the effect of altering pH, glucose, and osmolarity on the matrix production rates of nucleus pulposus (NP) cells.

METHODS: Goat NP cells were encapsulated in 1.5% alginate and ionically crosslinked with 100 mM calcium chloride for 20 minutes to form spherical beads at a seeding density of 2.5×10^6 cells/ml. Beads were cultured at 37°C in a humidified atmosphere containing 5% CO₂ and 5% O₂ in DMEM without glucose. Healthy and degenerate microenvironments were simulated by altering glucose (3mM and 1 mM), pH (7.1 and 6.8), and osmolarity (500mOsm and 300mOsm) levels. Beads were cultured with media changed every 3 days to maintain the microenvironmental conditions, as determined through in silico modelling (COMSOL Multiphysics). Beads were analysed after 14 days in culture for cell viability, DNA and GAG content, and using histology staining.

RESULTS: Preliminary data demonstrated that the GAG/DNA is altered by microenvironmental conditions in all cases. High osmolarity levels reduced the cell proliferation rate and consequently the matrix production rate of the cells. Variations in pH impacts the matrix production rate with a lower pH resulting in decreased matrix production. At higher glucose levels the glucose consumption rate is increased, and in silico models were adjusted to reflect this.

DISCUSSION & CONCLUSIONS: In this study, osmolarity was shown to impact the cellular behaviour significantly. To overcome this, beads will be cultured and primed prior to subjecting them to the relevant microenvironmental conditions thereby providing a protective niche around cells more closely mimicking the tissue substrate in vivo. This study has shown the microenvironmental profile of the IVD plays a vital role in the cellular response and is essential to the successful development of intradiscal therapies.

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Keywords: Intervertebral disc / spine and their disorders, In silico models

PP-400

Novel Mechanically Tuneable Three-Dimensional *In Vitro* Models for Prostate Cancer Progression and Adaptation under Different Stiffness

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INTRODUCTION: Prostate cancer is the most life-threatening cancer for men worldwide with high incident and mortality rates. The development of new early-detection biomarkers and therapeutics for prostate cancer have been hindered by the lack of preclinical systems resembling the human tumour microenvironment. Traditional two-dimensional (2D) cultured cells cannot recapitulate essential tumour characteristics such as cell-cell and cell-matrix interactions that are observed in tissues' three-dimensional (3D) organization. Additionally, 2D cultures cannot model the stiffness of prostate tissue, a property linked to disease development. To overcome these challenges, 3D culture models have been developed to enhance the complexity of in vitro models and provide a more realistic cell culture system.

METHODS: In this project, we have formulated alginate-based hydrogels to match the physical and chemical properties of the prostate microenvironments, in particular the stiffness and extracellular matrix composition. To develop this model, we used three prostate cell lines: the prostate epithelial cells PNT-2, the bone-metastatic prostate cancer cells PC-3, and the lymph node-metastatic prostate cancer cells LNCaP. Cells were encapsulated in alginate-gelatin-laminin hydrogel beads with different formulations. Soft hydrogels containing alginate (0.25% w/v), gelatin (3% w/v), and laminin (10 µg/mL) were designed to mimic physical properties of normal prostate microenvironment, whereas stiff hydrogels containing alginate (0.75% w/v), gelatin (3% w/v), and laminin (10 µg/mL) mimicked physical properties of prostate tumour microenvironment

RESULTS: Hydrogel stiffness was tested at different crosslinking density and set to 2.02 kPa for soft hydrogels and 6.01 kPa for the stiff ones. Prostate cells were encapsulated into hydrogel beads at an initial density of 1x10⁶ cells/mL; metabolic activity was measured up to 7 days. Whilst the metabolic activity of the prostate cancer PC-3 and LNCaP cells was reduced when encapsulated in stiff hydrogels, it was found to continuously increase in soft hydrogels. Conversely, the prostate epithelial PNT-2 cells did not show any change in their metabolic activity as function of hydrogel stiffness.

DISCUSSION & CONCLUSIONS: Our results show that prostate cancer cells behave differently in soft and stiff 3D microenvironments and this could be indicative of cellular adaptation. Detailed characterisation of relevant cellular biomarkers is needed to understand the cells' response to the altered microenvironment. The results of this study would provide novel insight into the prostate cancer cell adaptation to microenvironments. Further development and validation of this 3D in vitro model would help to understand the biochemical changes that occur during prostate cancer progression and could support the discovery of novel biomarkers and therapeutics.

Keywords: In vitro microenvironments, Cancer

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Development of Human Kidney Extracellular Matrix Hydrogels as 3D Platforms to Study Renal Fibrosis

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INTRODUCTION: Chronic kidney disease (CKD) represents a significant healthcare burden and is one of the leading causes of mortality worldwide. It can result from many different aetiologies, but irrespective of the origin, CKD is characterised by progressive tubulointerstitial fibrosis, ultimately leading to organ failure. The goal of this study is to develop a novel in vitro 3D human kidney hydrogel co-culture system to better understand the cellular and molecular mechanisms underlying fibrosis, and provide a platform for testing candidate anti-fibrotic therapeutics.

METHODS: Tissues from normal human kidney cortex (from donor kidneys unsuitable for transplantation) and fibrotic human kidney cortex (from explants of kidney transplants with chronic rejection and explanted because of graft rejection) were dissected into cubes and decellularised using sequential washes with water, sodium dodecyl sulphate, and DNase. Decellularisation was assessed by DNA content (DNeasy Kit, Qiagen), histology with haematoxylin and eosin (H&E) and picro-sirius red (PSR), and immunohistochemistry for extracellular matrix (ECM) proteins. To develop human kidney-ECM (hkECM) solution, decellularised cubes were homogenised, lyophilised and solubilised with pepsin. hkECM solution was mixed with nanocellulose (30% v/v; Cellink) to form stable hydrogels. Hydrogels were seeded with either a human renal proximal tubular epithelial cell (hRPTECs) line (HK-2), or primary hRPTECs, and cultured for up to 21 days. Viability and metabolic activity were assessed by Live/Dead and PrestoBlue® assays (ThermoFisher), respectively. Proliferation, apoptosis and hRPTECs phenotype were measured with Ki67, Cleaved-Caspase 3 and aquaporin-1 staining, respectively.

RESULTS: Kidney tissue was successfully decellularised as shown by a 97% reduction of DNA content. Histological analysis confirmed the elimination of nuclei by H&E staining, absence of cellular material (PSR); and collagen preservation (PSR). Maintenance of key ECM proteins (collagens I and IV, fibronectin, laminin α 5) was confirmed by immunohistochemistry. Combination of the normal or fibrotic human kidney cortex hkECM with nanocellulose produced stable hydrogels which supported HK-2 cells and primary hRPTECs as indicated by viability, metabolic activity, and proliferation, low apoptosis, and positive aquaporin-1 expression.

DISCUSSION & CONCLUSIONS: Normal and fibrotic human kidney cortex can be decellularised to develop hkECM solutions which, when combined with hRPTECs, generate human kidney 3D culture systems that support cell growth and proliferation. These models have the potential to recapitulate cell-ECM interactions in physiological and fibrotic conditions. This can provide insights into the role of the ECM in regulating cell differentiation and function, model tubulointerstitial fibrosis, and potentially establish a model for drug testing.

Keywords: Kidney, In vitro microenvironments

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Development of a completely defined inflamed human adipose tissue model

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INTRODUCTION: The increasing prevalence of obesity-related diseases like type 2 diabetes represents a significant societal health burden nowadays. Obesity correlates with inflammatory processes in adipose tissue having a systemic impact on all tissue types. Inflammation includes immune cell infiltration, especially of macrophages, and the release of pro-inflammatory mediators. Mechanisms of adipose tissue inflammation are frequently studied in animal models, yet their transferability to the human system is limited. Therefore, *in vitro* models resembling physiologically inflamed human adipose tissue are required to investigate fundamental processes of inflammation and their effects on other tissues. These *in vitro* systems must be reliable by avoiding interfering with animal-derived components such as collagen or serum. In this study, we developed a completely defined inflamed human adipose tissue model in static and perfusion culture as a promising future tool for pharmacological testing and basic research.

METHODS: The co-culture was set up of mature adipocytes (AC) encapsulated in 0.5 % gellan gum hydrogels floating in the defined medium and monocytes (MM6) seeded in the wells. These models were activated by phorbol-12-myristate-13-acetate (PMA) as a defined activator or lipopolysaccharide (LPS). For static culture, well plates were used, and for perfusion culture, the model was set up in a perfusion module connected to a pump with a flow rate of 200 μ L/h and cultured for 48 h. The viability of ACs and MM6 was determined by live-dead staining, and intracellular lipids were visualized by BODIPY staining. The cytokine response was measured by enzyme-linked immunosorbent assay for interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α . Morphological characteristics were analyzed by perilipin A of ACs and actin staining of ACs and MM6.

RESULTS: The co-culture exhibited high viability in the defined medium. It was possible to activate the co-culture with PMA in static and perfusion culture measured by a significant increase of cytokine concentration compared to unstimulated models. Co-cultured ACs exhibited a reduced lipid content and perilipin A integrity in reaction to PMA. In response to activation, MM6 showed typical membrane protrusions, whereas ACs showed no alterations of their cytoskeleton upon activation. Models in perfusion culture were comparable to those in static culture regarding viability and functionality, enabling more physiological culture conditions.

DISCUSSION & CONCLUSIONS: In conclusion, a completely defined inflamed human adipose tissue model was successfully established in static and perfusion culture. Further experiments continue to optimize the inflamed adipose tissue model by integrating adipose-derived stem cells and combining the model with other tissues like skin.

Keywords: Immunity / immunomodulation / macrophage, Biomaterials

PP-403

Novel in vitro Full Thickness Models of Human Epithelial Tissues and the Importance of Fibroblasts

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INTRODUCTION: Epithelial tissues are found throughout the body and are a component of larger structures such as the skin, intestine and oral mucosa. They share a common, highly conserved structure: a surface epithelium supported by a basement membrane and an underlying fibroblast-populated submucosal compartment. However, amongst different tissue types the structure of the surface epithelium differs and is highly specialised according to its function.

We hypothesise that signalling from the fibroblasts in the submucosal compartment is critical to the differentiation of the overlying epithelial layer. We propose to investigate whether these signals are tissue specific and assess the paracrine and juxtacrine influences between these cell populations.

The advent of advanced 3D tissue culture and tissue engineering technologies has enabled the production of complex, physiologically relevant in vitro tissue equivalents. We propose to test our hypothesis by developing models using tissue matched epithelial cells and fibroblasts in comparison to fibroblasts from different tissue types. We aim to determine whether tissue matched fibroblasts are required for the specialised differentiation of the epithelial layer or if generic fibroblasts from a common source can enable appropriate differentiation.

METHODS: Reconstructed human full thickness skin equivalent models have been developed utilising primary commercially available epidermal and dermal cells. Oral mucosal and intestinal models have also been developed using primary cells and existing cell lines. In each model, fibroblasts are seeded in Alvetex® Scaffold well inserts followed by epithelial cells seeded on top of this construct, which subsequently differentiate.

RESULTS: Our respective tissue models demonstrate structural morphology resembling the structure of native skin, intestine, and buccal mucosa. Fibroblasts produce endogenous extracellular matrix proteins which provides support for the overlying epithelia. Extensive characterisation of the mucosal models shows evidence of basement membranes and multiple other structural and functional features shared by the corresponding real tissue. When epithelial cells were cultured with non-tissue matched fibroblast sources this had a significant influence on the differentiation and morphology of the overlying epithelia. Intestinal epithelial cells showed greater polarisation. Oral epithelial cells underwent a more epidermal-like differentiation pathway to produce a terminally differentiated layer. Presence of a stratum corneum-like structure suggests plasticity and the capacity of the buccal epithelial cells to transdifferentiate resulting in a more skin-like morphology when cultured with dermal fibroblasts.

DISCUSSION & CONCLUSIONS: Our data provide preliminary evidence that tissue specific fibroblasts are required for the formation of fully differentiated mucosal tissue constructs and alternative fibroblasts sources can alter epithelial cell differentiation.

Keywords: Microenvironment and niche engineering, Differentiation

PP-404

Friend or foe? Transglutaminase-2 and a complex role in the tumour microenvironment

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INTRODUCTION: Development and progression of cancer is dependent on complex interactions between cancer cells and the tumour microenvironment (TME), which lead to extensive tissue remodelling. Excessive deposition and enzymatic modification of matrix components create a stiff, fibrous microenvironment, driving cancer progression through biomechanical signalling¹.

Transglutaminase-2 (TG2) is a protein cross-linking enzyme prominently expressed in the TME and linked to progression of a range of cancers. Paradoxically, TG2 appears to restrict tumour spheroid growth in vitro through matrix cross-linking - yet TG2 in patients is linked to poorer disease outcome². To investigate the complex role of TG2, this work examined how cellular communication within the TME determines TG2 expression in colorectal cancer (CRC), and how this links to invasive behaviour and treatment response in a range of in vitro TME models.

METHODS: SW480 CRC cells were co-cultured with MRC5 fibroblasts to generate spheroids by embedding in collagen-based hydrogels (JellaGel, Col-Tgel) yielding distinct stiffness matrixes. Decellularised colorectal tissue was recellularised with SW480 and MRC5 cells. Gels and recellularised tissue were incubated with recombinant TG2 or TG2 inhibitors for 7-14 days of culture and probed with doxorubicin to monitor drug penetration by live imaging fluorescence. Gels and recellularised tissue were then fixed, paraffin embedded and sectioned for analysis. Haemotoxylin/eosin and masson's trichrome staining were performed. Protein expression analysis was performed by Western blotting for TG2.

RESULTS: TG2 expression was prominently expressed around the invasive edge of tumour spheroids. Inhibiting TG2 altered CRC spheroid shape and increased uptake of doxorubicin.

DISCUSSION & CONCLUSIONS: TG2 appears to limit bioavailability of chemotherapy agents in spheroids by restricting to its periphery. We propose that short-term restriction of tumour growth may come at the cost of reduced treatment efficiency with implications for patient treatment and outcome.

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Keywords: Microenvironment and niche engineering, Drug delivery

PP-405

3D flow-focusing microfluidic biofabrication of complex biomimetic architectures, from in-silico to in-vitro

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INTRODUCTION: The microfluidic manipulation of hydrogels is an efficient method for simulating functional biological architectures. We used a single 3D hydrodynamic flow-focusing chip to generate a continuous flow of hydrogel precursors that reorganize based on viscosity and applied pressures (1). These crosslink into fibers with a variety of new multi-compartment shapes down to yet unreported minimal dimensions, allowing for the recreation of a broad range of complex biological architectures and micro-modeling of distinct 3D environments.

METHODS: Gellan Gum, Alginate, and GelMA were studied as hydrogel precursors to generate multi-composition microfibers utilizing a single flow-focusing microfluidic chip for continuous wet-spinning. We modeled the system in-silico and tweaked precursor viscosity and inlet channel organization to change the 3D arrangement of fiber compartments. We obtained core-shell vascular-like structures and networks and ribbon-like cancer/basement-membrane/stroma models.

RESULTS: We created human dermal microvascular endothelial cell-laden fibers with core-shell morphologies. By pulling the fiber at a greater rate than it is spinning, a significant size decrease may be achieved without compromising the distribution of compartments inside the fiber. We created entire core-shell fibers with a total diameter of less than 50 μ m using this method. Furthermore, we were able to control the flow conditions by applying sinusoidal pressure (increasing or decreasing in time), demonstrating that it is feasible to generate, for example, a fiber with a constant diameter but a progressively growing core-to-shell size ratio. Cells in the degradable core migrated to the core-shell interface, where they reorganized forming an empty lumen, generating a structure capable of modeling tissue vasculature and integrating into 3D tissue settings. Ribbon-like shapes were found to be capable of integrating two cellular compartments (cancer melanoma cells and human stromal fibroblasts) separated by a thin basement membrane. This structure was used to study cancer cell invasion in vitro, where the basement-membrane-like proteins can impact cancer cell migration and response to treatment. The response to doxorubicin was discovered to be dependent on the model's complexity and the presence of fibroblasts exerts a protective effect on the doxorubicin-induced cancer cell death.

DISCUSSION & CONCLUSIONS: The microfluidic-driven biofabrication of hydrogel fiber constructs is a widely adaptable and versatile technology that can provide unprecedented possibilities for miniaturizing and modeling complex biological events.

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Keywords: Biofabrication, In vitro microenvironments

PP-406

Analysis of various 3D human adipose tissue models compared to 2D monolayers

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INTRODUCTION: Adipose tissue secretes hundreds of important cytokines that influence crucial body mechanisms like food intake, blood pressure, and immune system activation. An altered state of the cells (e.g. overweight) within adipose tissue leads to an altered cytokine profile which in turn affects the body's homeostasis and leads to the development of severe diseases like diabetes or cardiovascular disorders. The wide-ranging secretory activity of adipose tissue underlines the importance of reliable human-based in vitro models for research purposes

METHODS: Therefore, four different 3D in vitro models based on adipose-derived stem cells (ASCs) and mature adipocytes (ACs) were established, and the cell state during adipogenesis and maintenance was compared to ASCs seeded on tissue culture polystyrol (TCPS). The 3D models encompass ASC spheroids, ASCs in 1 % gellan gum (GG)-hydrogels, ACs in 0.5 % GG-hydrogels, and ex situ adipose lobule

RESULTS: During adipogenesis, ASCs in all model types accumulated lipids (lipid staining) and changed their morphology (cytoskeleton visualization) to multivacuolar cells. The successful differentiation was also demonstrated by the upregulation of PPAR γ and CEBP α , which was the highest in ASC GG-hydrogels. All four 3D models and the TCPS culture were analyzed during the maintenance phase. The cells within AC GG-hydrogels and ex situ lobule showed an univacuolar morphology right from the beginning, which was preserved during the three weeks of maintenance. For the ASC-based models, the cells within the GG-hydrogels and in spheroids partly fused to univacuolar cells, as shown by lipid perilipin A, and actin staining. Only the TCPS culture showed multivacuolar cells during the whole time. In summary, comparing the different models has demonstrated that the cells exhibited distinct behaviors regarding morphology, size, and function, as also shown in the RT-qPCR.

DISCUSSION & CONCLUSIONS: In this work, we successfully established various tissue models from different primary cells from human adipose tissue. Comparison has shown that, depending on the cell type and model, the functional maximum is at another point in time, and thus, the comparability to the native state varies. This suggests that the models should be chosen according to the research question and the intended purpose

Keywords: In vitro microenvironments,

PP-407

In vivo assessment of marine vs bovine origin collagen-based composite scaffolds promoting bone regeneration in a New Zealand rabbit model

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INTRODUCTION: Allografts, artificial bone grafts, and, particularly, autografts, are still the golden standard treatments for critical-size bone defects, but limitations like donor tissue availability, morbidity, and ultimately, the poor efficiency of these treatments have been touching the scientific community to investigate alternative routes. Collagen-based 3-dimensional (3D) engineered structures stand out as promising biomaterials since they offer attractive biological cues that encourage cell and tissue integration in vivo with reduced inflammatory response and represent biodegradation and bioresorbability capabilities.

METHODS: Collagen extracted from blue shark skin through an acidic method [1] was combined with bioapatite obtained from blue shark's teeth (mColl:BAp), while collagen extracted from bovine hides following the same methodology and combined with synthetic hydroxyapatite (bColl:Ap), to produce 3D composite scaffolds by freeze-drying [2]. Scaffolds were characterized, and the in vivo performance regarding bone tissue regeneration was evaluated in critical-size femoral condyle defects created in New Zealand rabbits. Twelve weeks post-surgery the results were evaluated by micro-CT and histomorphometry.

RESULTS: Marine and bovine collagen revealed a similar profile, while the bioapatite and the synthetic apatite particles differed in its composition, being bioapatite a fluoride-enriched ceramic. Both scaffolds formulations presented high porosities, being the marine origin scaffolds more porous. In addition, bioapatite reinforced fish collagen scaffolds displayed a higher compression modulus when compared with synthetic apatite reinforced bovine collagen. Micro-CT analysis of the retrieved condyles showed that the implanted mColl:BAp scaffolds had a slower degradation and promoted a higher tissue formation (17.9 ± 6.9 %), when compared with bColl:Ap scaffolds (12.9 ± 7.6 %). The histomorphometry analysis corroborated the observed tendency, determining 13.1 ± 7.9 % and 10.4 ± 3.2 % of new tissue formation for mColl:BAp and bColl:Ap composites, respectively.

DISCUSSION & CONCLUSIONS: The present findings revealed that both composite structures promoted bone-like tissue formation, suggesting an improvement when compared with the control group (9.6 ± 6.3 % by micro-CT and 6.8 ± 4.3 % by histomorphometry), although without statistical significance. As bovine collagen and synthetic hydroxyapatite are well established materials in bone tissue engineering, the observed outcomes highlight the potential use of marine origin biomaterials

for bone regeneration.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-408

Kainic acid excitotoxicity induced chemical Spinal cord injury in vivo model for assessment of neuroregeneration potential of drugs

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INTRODUCTION: Glutamate induced excitotoxicity following spinal cord injury (SCI) play a main role in neuronal cell death and nerve degeneration. Kainic acid (KA) is a non-degradable glutamate analogue that is 30 times more potent in inducing neuropathy than glutamate. This study aimed to induce moderate paraplegia via intraspinal injection of KA in Sprague-Dawley rats and to study the utility of the in vivo model in nerve regeneration evaluation.

METHODS: A 40 µL of 0.05 mM KA was injected intra-spinaly at the rate of 0.01 mL (10 µL)/min at intravertebral space between T12-T13 vertebrae. The animals were then observed for locomotor, electrophysiological & pathological assessments in injured (I), treated (T) & healthy (H) group at subsequent days (3rd, 7th, 14th, 21st & 28th). T group was treated orally with proprietary agent for 4 weeks starting at Day 0 of the injury, and were assessed for a potential neuro-regenerative treatment. The Somatosensory evoked potentials (SEP) potential was also recorded at pre-injury, post-injury (within 30 min), 14th & 28th day to observe any delay in latency and amplitude in conduction.

RESULTS: All animal completely lost lower limb movement until 7th days, then slowly started regaining movement and balance. The T group re-gained balanced movement within 2 weeks; while I group took 3 weeks to re-gained balanced movement and the differences were statistically significance ($p < 0.05$). The electrophysiological recording showed improved waveform in T group at 14th day, showing treatment helped to recover lost connection. The immunocytochemistry (IHC) staining showed higher expression of GAP43 in T group.

DISCUSSION & CONCLUSIONS: KA caused impaired hind limb motion, and it is a successful laboratory mean to induce SCI & the proprietary drug was found efficient as a potential treatment promoting neuro-regeneration.

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Keywords: Disease models, Enabling technologies

PP-409

Modeling peritoneal adhesions: mechanics meet systems biology

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INTRODUCTION: Peritoneal adhesions are pathological fibrotic connections forming between organ surfaces. They typically develop following a surgical procedure of the thorax, abdomen or pelvis, or result from inflammatory diseases. Peritoneal adhesions can cause small bowel obstruction, chronic pain or infertility and are identified nowadays as a major economic and health burden. However, the underlying mechanisms of tissue physiological repair and adhesion formation are still poorly understood [1]. The role of biomechanics is critical to provide better understanding, prevention and treatments for peritoneal adhesions, especially regarding pain management. Indeed, nociception and mechanics are strongly connected [2]. We present a novel model of peritoneal adhesions depicting the main biochemical mechanisms associated with adhesion formation and their mechanical consequences.

METHODS: We propose here a 2D model of adhesion formation representing the volume between the peritoneal membrane and the tissue boundary, where the following mechanisms occur: (i) the formation of the fibrin exudate upon tissue insult, (ii) fibroblast-mediated fibrosis and (iii) cyclic loading simulating typical breathing boundary conditions.

In particular, we investigate here the evolution of main biochemical agents responsible for material properties of the medium following trauma: fibrin - the principal element in the clot formation during peritoneal wound healing; tissue plasminogen activator (tPA) - a potent coagulation agent; fibroblasts - responsible for fibrosis and membrane regeneration; collagen, the main structural element of adhesions.

We implemented a system of partial differential equations depicting the time- and space- dependent evolution of fibrin, tPA and fibroblast concentrations, accounting for fibrin-mediated chemotaxis of fibroblasts in the peritoneal fluid. Additionally, the evolution of collagen content was derived from fibroblasts activity and collagen degradation. In order to simulate mechanical solicitations of the breathing cycle, we imposed a cyclic displacement of the injured serous membrane.

RESULTS: In the case of a healthy repair, the inhibition of fibrin by tPA-mediated fibrinolysis prevents formation of the dense connective tissue, thus preserving the mechanical behaviour of the system. Instead, with low tPA local production, the fibrin clot develops into a dense collagenous gel binding to the neighboring membrane. As expected, this induces larger strains within the injured and the healthy tissue.

DISCUSSION & CONCLUSIONS: This is the first attempt to quantify the mechanisms of adhesion formation. We successfully depicted the main events following tissue trauma. Future work will investigate the next remodelling steps, in particular involving innervation and *in vivo* mechanical stimulation.

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Keywords: Wound healing, Trauma / surgery and rehabilitation

PP-410

Using a machine learning-supported approach for assessing and predicting articular cartilage surface damage and its susceptibility to mechanical injury-induced cell death and survival

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INTRODUCTION: Accurate articular cartilage (AC) damage evaluation remains difficult in clinical practice but may help in the future to direct treatment choices. In this context, our group showed recently that the spatial organization of superficial chondrocytes (SCSO) can be used quantitatively as a surrogate marker of the loss of the articular surface nanoscale stiffness and for predictive modelling. Here, we asked whether the SCSO can be used as quantitative marker of AC surface damage and cell survival after injury.

METHODS: We performed scanning electron microscopy (SEM) of 18 human osteoarthritic (OA) cartilage explants, used the SEM images for AC surface texture analyses on the level of entire samples and individual image tiles, and chose local entropy as marker of damage. Fluorescence microscopy of the same samples allowed co-localizing entropy and SCSO. We applied mechanical injury on a second set of AC samples, evaluated the local cell survival rate, and quantified the SCSO. Multivariate analyses and machine learning were used to discriminate AC samples with no/minimal damage and to predict cell survival after trauma, using the SCSO as input.

RESULTS: AC surface damage was quantifiable as surface texture entropy of image tiles. Entropy correlated significantly with the SCSO type and the graded macroscopic AC surface damage ($p < 0.01$). The quantified SCSO (qSCSO) was significantly different in minimally vs. highly damaged AC tissue regions ($p < 0.001$), which were then classified in a prediction model trained on qSCSO parameters. This enabled accurate AC damage assessment in 83%. We then asked how many local image tiles are needed that represented the entire data set and used a support vector machine learning algorithm. This revealed that the minimum number of image tiles was $n=4$ that was needed for accurate classification of representative images.

In the second part, we demonstrated that the extent of cell death and survival after injury depended on the SCSO type, as more cells died in advanced SCSO stages, and that cell survival was predictable in a classification model (82%).

DISCUSSION & CONCLUSIONS: AC surface damage and post-injurious survival can principally be assessed and predicted when using the SCSO as surrogate marker. Extending this capability towards a clinically usable technology might help to advance clinical diagnostic capabilities and early disease diagnosis.

Keywords: Omics / bioinformatics and systems biology, Cartilage / joint and arthritic conditions

PP-411

BEMSCA: A Novel Decision Support Tool for Optimally Designing Large-Scale Stem Cell Expansion Bioprocesses

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INTRODUCTION: Stem cells have long been poised to revolutionise the treatment of numerous diseases due to their inherent ability to self-renew and to differentiate into distinct cell types. However, despite great efforts, they have yet to become part of regular clinical practice. To a large degree, this is due to the lack of sufficiently consistent and cost-effective bioprocesses for producing the large cell numbers required for potential therapies. Therefore, tools which aid the design and development of such bioprocesses are of unquestionable importance.

METHODS: A computational tool named Bioprocess Economic Model for Stem Cell Applications (BEMSCA) was developed using Python. BEMSCA performs Monte Carlo simulation, based on biological variables and logistical considerations, to determine the optimal workflow which produces a desired cell number, along with its cost and duration. In computing the optimal workflow, BEMSCA minimizes costs while taking into account the biological probability of achieving the target cell number. To showcase its applicability, BEMSCA was employed to simulate a bioprocess that sought to produce 1.5×10^9 hiPSCs starting from 1.0×10^6 hiPSCs using vertical-wheel bioreactors. Three promising strategies were then assessed, namely dextran sulfate (DS) supplementation, low-density inoculation and substitution of mTeSR1 with B8, in an attempt to reduce cost and duration.

RESULTS: BEMSCA estimated that achieving the target cell number with the default bioprocess would cost €32,220 and take 43 days. When simulating the same bioprocess with DS supplementation, BEMSCA predicted that the cost and duration would be €19,820 (38% less) and 30 days, respectively. For low-density inoculation, the cost and duration were estimated as being €22,830 (29% less) and 29 days, respectively. Substituting mTeSR1 with B8 could reduce the cost to as little as €13,590 (58% less) and duration to 30 days.

DISCUSSION & CONCLUSIONS: All of the simulated strategies led to a remarkable projected decrease of bioprocess costs. The decreased costs of DS supplementation and low-density inoculation can be attributed to improved hiPSC fold expansion, while the main impact of substituting mTeSR1 with B8 is eliminating the culture medium's role as the major cost driver. This study highlights the value of tools such as BEMSCA when it comes to bioprocess optimisation.

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Keywords: Scaling up & manufacturing, Induced pluripotent stem cells

PP-412

Co-occurring changes in single cell morphology and cytoskeletal F-actin intensity, density and distribution under various regimens of cyclic stretch

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INTRODUCTION: A range of studies has investigated the effects of mechanical stretch amplitude and frequency. However, how low vs. high levels of stimulation or one- vs. or two-times application of stimulation affect cell morphology and cytoskeletal F-actin remain unknown. Such insights would be helpful for utilizing mechanical forces for controlling cell shape and function.

METHODS: Hela cells were seeded onto Fibronectin-coated polyacrylamide hydrogels and cyclically stretched (incubator-housed ElectroForce-5210-BioDynamic System). Twelve experimental groups were used: low stimulation consisted of 10% stretch applied for 1 and 2.5h ± break times, high stimulation consisted of 10% stretch applied for 16-40h ± break times; both regimes were applied one or two times. Intensity-calibrated immunofluorescence and trainable segmentation were used for measuring single cell morphology (area, length, width, circularity, aspect ratio, roundness, solidity), F-actin intensity and density, and stress fiber amount and entropy. Statistics were performed with SigmaPlot.

RESULTS: Mechanical stimulation had a significant impact on almost all of the measured cell shape descriptors and on F-actin intensity, density, and stress fiber distribution. In the low stimulation group, the descriptors solidity, roundness, aspect ratio, and cell length were significantly changed ($p < 0.05$) but the changes were generally small. In the high stimulation group, and especially when applied two times, those single cell shape changes were significant ($p < 0.05$) and much larger, compared to low stimulation. The maximum effect was found under high stimulation, which produced the largest and longest cells ($p < 0.05$). The stretch-induced changes in F-actin intensity and density were significant for almost all stimulation groups ($p < 0.05$) but these changes did not follow the pattern observed for cell area and length. High F-actin intensity and density values occurred at the beginning of short stimulations and the end of longer stimulations. Analyses revealed in each stimulation group multiple significant ($p < 0.05$) but weak correlations between shape descriptors and F-actin intensity and density in the low stimulation group. In contrast, there were many significant, strong correlations in the high stimulation group ($p < 0.05$). When testing for significant correlations across the entire data set, single cell area and length correlated with F-actin intensity, density and stress fiber amount and entropy ($p < 0.0001$). However, stress fiber amount and entropy correlated with the F-actin density ($p < 0.001$) but not intensity.

DISCUSSION & CONCLUSIONS: Mechanically induced alterations of single cell morphology co-occurred with F-actin network changes but not in a one-to-one relationship.

ACKNOWLEDGEMENTS: Haibin Zhang and Ruiqiang Wang have a shared co-first authorship.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, In vitro microenvironments

PP-413

GelMA hydrogel mechanics affect the collective migration of fibroblast cells

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INTRODUCTION: Collective cell migration is an important event that occurs in morphogenesis, regeneration, and cancer invasion [1,2]. GelMA is a bioactive hydrogel that mimics 3D extracellular matrix in tissues and organs with mechanical tunability to study the mechanobiological impact of cell-matrix interaction.

METHODS: We studied the impact of mechanical stiffness on collective cell migration. We cultured mouse embryonic fibroblasts in soft (0.5%), medium (2%), and stiff (10%) hydrogels of GelMA for 7 days in DMEM high glucose medium with 10% FBS and 1% penicillin-streptomycin antibiotic. After 7 days, the gel was fixed in 4% paraformaldehyde and the actin cytoskeleton was labeled using Phalloidin conjugated with Alexa-Fluor 647. The cells were imaged label-free with DIC, and the actin cytoskeleton was imaged with a line confocal microscope. The directionality of the actin fibers was analyzed in the Fiji plugin OrientationJ [3].

RESULTS: We observed that cells do not have any directionality during group spread in the soft matrix. In the medium matrix, however, the cells showed the highest directed alignment in spread with the multiple bundles propagating with different heads. On the other hand, the cell spread was more sheet-like in the stiff matrix. In terms of the number of cytoskeletal actin orientations, we found that the medium stiffness matrix had the lowest number of angular directionalities compared to both soft and stiff matrices, with the soft matrix demonstrating the highest directional variations. This implies that the medium stiffness was more aligned in a specific direction compared to both soft and stiff matrices.

DISCUSSION & CONCLUSIONS: The study suggests that the selection of an optimal mechanical stiffness can stimulate cells to attain suitable directionality features for collective cell migration with respect to a target structure such as tissues in organs with potential implications in repair, morphogenesis, and regenerative medicine.

ACKNOWLEDGEMENTS: This work was supported by H2020 funded FET-Open RIA project OrganVision (id 964800).

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Keywords: Biomaterials, Hydrogels and injectable systems

PP-414

Application of controlled multidirectional hydrodynamic stimuli on human endothelial cells in a novel bioreactor

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INTRODUCTION: Atherosclerosis is a chronic-degenerative occlusive disease. Alteration of the blood flow is a main pathogenic risk factors, acting on mechanotransduction mechanisms. Vascular endothelial cells (EC) are extremely sensitive to tangential stress occurring in the vessel wall (wall shear stress, WSS), related with local changes of fluidodynamics. It is known that the arterial districts are mostly interested by disturbed fluidodynamics. Altered WSS is a trigger of endothelial dysfunction and permeability increase. Despite this, the relationship between the hydrodynamic stress sensed by EC and its effects on activation of EC themselves has not yet been fully understood. In this scenario, we have developed a novel device allowing to apply multidirectional, complex WSS patterns in controlled conditions for investigating the effects of specific WSS on vascular EC behaviour.

METHODS: The strategy chosen for the bioreactor design is to apply a constant flow rate while the sample rotates with a specific motion function. In this way it is possible to reproduce the WSS multidirectional nature sensed in vivo by EC. The designed bioreactor consists of a vascular sample housing support to be inserted in a parallel plates flow chamber. Thanks to the presence of two gears, it is possible to transfer the rotary motion of a stepper motor to the entire housing system and consequently to the biological sample. The motor's movement and the fluidic circuit are controlled by a custom developed electronic interface, for setting and monitoring the experimental parameters. The device was successfully verified by performing bench tests: in silico simulations and water tightness tests. Preliminary experiments (n=3) applying atherogenic and atheroprotective WSS over EC (primary human aortic EC, 250000cells/mm²) plated for at least 5days on uncoated supports were also performed. Cell viability and distribution were assessed at fluorescence microscope upon labelling with Acridine Orange dye (670nM).

RESULTS: Preliminary data applying atherogenic WSS showed a local disorganization of the living ECs over the support and/or the detachment of some EC, whereas atheroprotective WSS led to a tendency of EC to acquire spindle shape and orientate along with the flow direction.

DISCUSSION & CONCLUSIONS: In conclusion, the prototype represents an innovative device with respect to the current state of the art enabling to investigate the WSS effects. In perspective the device will be tested on co-cultures or vascular tissue samples to examine the complex mechanobiological interactions that occur in the subendothelial tissue in the early stage of the pathology evolution.

Keywords: Bioreactors, Cardiovascular

PP-415

Engineered Cancer *In Vitro* Model To Demultiplex Biophysical Cues In Metastasis

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INTRODUCTION: Prostate cancer is among the most common cancers affecting men worldwide, with 17–34% of cases diagnosed at advanced stage, requiring new models to help understand disease progression. Viscoelasticity has been widely identified as a mechanical property of interest involved in the cell-extracellular matrix interaction which is deregulated during metastases.[1,2] Developing materials that can independently tune viscoelasticity, within a range mapped to that of patient material, is key to further investigating this effect. To directly compare human tissue samples and *in vitro* models, a method of analysing the rheological properties with respect to viscoelasticity is required.

METHODS: To develop a mathematical model to analyse viscoelasticity, the different sections of viscoelastic behaviour during a creep-recovery cycle[2] were identified. The behaviour of each section can be modelled as a spring (fully elastic), dashpot (fully viscous), or combination of the two (time-dependant response). By combining the Maxwell (fully elastic and viscous) and Kelvin (time-dependant response) models, the Burgers model is achieved, quantifying the elastic, viscous, and viscoelastic elements, and can be altered to match different sample types. This model was then tested on data from healthy prostate samples to verify how accurate and reliable the fit is.

RESULTS: A model has been developed that receives raw data from mechanical tests (time and strain) and fits the data, producing values for the elastic, viscous, and viscoelastic elements of the sample. This also calculates the relaxation over time, to be compared between the human tissue and gel samples.

DISCUSSION & CONCLUSIONS: Relaxation of the substrate is an important parameter in understanding the viscoelastic effect during cell-substrate interactions.[3] Initially, the model will be used to better understand how varying the formation of the gel effects the viscoelasticity. Once a small library of alginate-based gels has been produced, where viscoelasticity can be varied independently, the effect of different relaxation rates on the cell-substrate interaction can be assessed. This will aid in developing understanding on the role of substrate viscoelasticity in cellular behaviour, as well as the impact that mechanical changes have during disease progression.

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Keywords: In vitro microenvironments, Biomaterials

PP-416

The role of heterogeneous nuclear ribonucleoproteins in mechanosensing

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INTRODUCTION: Heterogeneous nuclear ribonucleoproteins (hnRNPs) belong to the group of RNA-binding proteins. Their main function is to contribute to pre-mRNA processing and transport. Twenty major types of hnRNPs exist that are named alphabetically. It has been previously shown by our group that the intracellular localization and function of RNA binding protein, hnRNPC, can be mechanically altered during ECM pathological remodelling in the diseased heart (Martino et al, 2022).

METHODS: Here we employed micropatterned surfaces controlling induced pluripotent stem cell (iPSC) colonies shape and size to demonstrate that hnRNPA1, hnRNPA/B, hnRNPC, and hnRNPU acquire a unique polarized distribution at the nuclear border of the iPSCs facing towards the free-space. This area is known to be exposed to higher mechanical stress than the center of the colony (Muncie et al, 2020). Next we asked what the function of the hnRNPs might be at this site and used antibodies against acetylated or methylated forms of histone H3 to show that hnRNPs colocalize with euchromatin regions.

RESULTS: It has been previously shown that mechanical compression of cells induces chromatin condensation, while stretching leads to chromatin decondensation (Hsia 2022). Also, we performed scratch assay to demonstrate hnRNP polarization emerges within few minutes after creating a new edge in iPSC colonies. This suggests that the phenomenon is a result of protein relocation within the nucleus, not primarily due to expression changes. Interestingly, hnRNP nuclear polarization was observed also in iPSC 3D spheroids, where the interaction with the ECM does not play any major role.

DISCUSSION & CONCLUSIONS: Given that hnRNPs form a complex structure at the periphery of the nucleus in edge cells in iPSC colonies, revealing the composition of these bodies will help to elucidate their specific mechanosensitive function in correspondence with the open chromatin.

ACKNOWLEDGEMENTS:

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Stem cells - general

PP-417

Guiding engineered bone tissue growth using mesostructured surfaces of defined geometry

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INTRODUCTION: Most cells respond to geometrical cues in their environment. Within the context of bone, several studies have revealed that extracellular geometry strongly influences spatiotemporal patterning of bone (progenitor) cells, which is attractive for tissue engineering. However, most studies involved relatively simple geometric environments, and only focussed on spatial cell patterning, leaving many questions of geometry-driven tissue growth unanswered. Here, we combine mathematical design, high-resolution fabrication, and multimodal characterisation to study de novo bone tissue growth on more complex geometries, focusing on the spatiotemporal organisation and mineralisation of the extracellular matrix (ECM).

METHODS: Mesostructured substrates (10^1 - 10^3 μm) with defined surface curvature were designed using a custom parametric tool in Matlab. Substrates were fabricated using two-photon polymerisation printing, followed by replica moulding in polydimethylsiloxane (PDMS), polystyrene (PS) or polycaprolactone (PCL) and coating with fibronectin. Human mesenchymal stem cells (hMSC) or murine preosteoblasts (MC3T3-E1) were cultured on the substrates for several weeks under varying osteogenic conditions. Following fixation, the cells were fluorescently stained (e.g. F-actin, hydroxyapatite, fibronectin) and imaged using 3D confocal microscopy and second harmonic generation imaging (SHG). Volumetric Raman microspectroscopy and scanning electron microscopy were employed to obtain 3D biochemical maps and additional structural information. Samples were also stained using Alizarin Red (ARS) for brightfield microscopy and absorbance-based quantification. The imaging-based datasets were quantitatively post-processed using Fiji (e.g. OrientationJ) and custom Matlab and Python codes.

RESULTS: Both cell types demonstrated a clear avoidance of convex geometries and a preference towards concave regions. Highly stretched cells were observed spanning large concave domains, aligning longitudinally in channel-like geometries, and effectively smoothening the underlying substrate geometry. SHG revealed a clear collagen network from day 14 onwards, showing strong deposition and alignment in concave channels that correlated with local diameter, and no preferred alignment on planar or convex regions. ARS revealed significantly higher calcium deposition on structured domains as opposed to planar regions. Raman and confocal imaging further demonstrated spatial differences in hydroxyapatite deposition, with nodules preferentially forming in channel-like geometries with dense collagen matrices.

DISCUSSION & CONCLUSIONS: Our results confirm a clear role for geometry in spatiotemporal cell patterning and ECM formation, even in complex environments with varying curvatures. Local channel-like regions induce strong and dense collagen alignment, which correlates with enhanced mineralisation. Moreover, our results confirm a tight coupling between extracellular geometry and mechanical forces during tissue growth. These insights are fundamentally relevant for biomineralisation, and could inspire the geometric design of tissue engineering scaffolds.

Keywords: Bone and bone disorders (osteoporosis etc), Microenvironment and niche engineering

PP-418

3D tissue formation increases intracellular macromolecular crowding which programs the metabolism and lineage commitment of stem cells

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INTRODUCTION: Three-dimensional (3D) cell culture systems are thought to mimic the native cellular environment better than conventional monolayer cell culture. However, how 3D cell culture systems affect intracellular processes leading to the more physiologically relevant behaviour has remained largely unclear. Chondrogenesis is known to be enhanced by aggregate culture [1], hence we investigated how aggregate culture itself causes intracellular changes to facilitate chondrogenesis. **METHODS:** Human periosteum derived stem cells (hPDCs) were seeded in agarose microwell inserts, containing microwells of 200 µm [1], at a seeding density of about 100 cells per microwell. The resulting aggregates were cultured in either proliferation medium or chondrogenic differentiation medium. Inhibition studies were performed in both media. Aggregate size, cell size, and cell volume were investigated using brightfield and confocal imaging. Intracellular crowding was determined with flow cytometric and holotomographic microscopy analysis. Cellular metabolism was assessed with Seahorse assays. FOXO1/3A levels were determined with RT-qPCR, Western blot, and immunostaining. Mitochondria were assessed with MitoTracker staining and TEM imaging. Artificial crowding was performed with PEG300 or salt.

RESULTS: Culturing hPDCs in aggregates led to a decrease in cell volume and an increase in intracellular crowding. This volume decrease could be mitigated via exposure to metabolic blockers, cytochalasin D, and FOXO1 inhibitor. Interestingly, we found that aggregate culture in proliferation medium upregulated pro-chondrogenic transcription factors FOXO1 and FOXO3A. Also, both glycolysis and oxidative phosphorylation were decreased in aggregate cells as compared to monolayer cells. The decrease in oxygen consumption rate could be emulated in monolayer cells by artificial crowding. Moreover, mitochondria were more densely clustered in aggregate cells, which could also be mimicked in monolayer cells by artificial crowding.

DISCUSSION & CONCLUSIONS: We reveal that aggregate culture changes several key intracellular processes known to influence cell behavior and lineage commitment. Both intracellular crowding and cellular metabolism are known to influence a myriad of cellular processes. Notably, we identified transcription factors FOXO1 and FOXO3A as lead candidates to augment chondrogenesis via master transcription factor SOX9 [2]. This study therefore reveals a new mechanism via which the third dimension controls cellular function.

ACKNOWLEDGEMENTS: Financial support was received from Dutch Research Council (Vidi, 17522) and European Research Council (Starting Grant, 759425).

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Keywords: Stem cells - general,

PP-419

Tendon stem cell populations have distinct proliferative and secretome profiles - a key role for physiological oxygen

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INTRODUCTION: Tendon injury is a common problem affecting lives and occurs most often during sports¹. Traditionally, tendons were considered to only contain tenocytes (TNCs), but recently tendon stem cells (TSCs) have been identified and classified into TSCs I (sheath) and TSCs II (fibres)². Extracellular vesicles (EVs) are cell-produced signalling packages that can alter cell function. Tendon cell-derived EVs treatment has been proposed as a potential regenerative strategy to improve repair outcomes³.

METHODS: Cells were isolated from a porcine Achilles tendon through either enzymatic digestion (TSCs) or explant method (TNCs). Culture was performed in both 21% and 2% oxygen conditions. Characterization was performed via flow cytometry, immunofluorescence (IF) for tenomodulin expression, and molecular analysis for tenocyte-specific and stem cell markers. Multipotency was explored through quad-lineage differentiation at different passages to test osteo/adipo/chondro/teno-genesis.

CM was collected from TSCs II and TNCs. Briefly, when cells reached 80% confluence, serum-free medium (SFM) was added to cells for '24h-conditioning' and capturing the secretome, including EVs. CM was characterized by protein quantification (Bradford and quantitative cytokine analysis) and image analysis (SEM and TEM). The tenogenic effect of CM (and their constituent EV components) was then explored on TSCs II in the presence of pro-tenogenic GDF5. Cells were then characterized by IF and transcript analysis.

RESULTS: TSCs II displayed enhanced proliferation when compared to both the others. Flow cytometry analysis confirmed the expression of the stem cell surface markers: CD44 and CD90. The multi-lineage differentiation potential of tendon cells was confirmed by histology, IF, and molecular analysis confirmed the expression of all markers, which were higher expressed in 2% O₂. CM protein quantification showed that after centrifugation there was a minimum of 5-fold decrease in protein-amount. Cytokines analysis showed that TSCs II CM in 2% O₂, only, expressed all those measured. Morphological analysis indicated sub-200nm diameter vesicles, measured through TEM and SEM, as potential EVs.

The tenogenic effect of CM was confirmed by tenomodulin IF and molecular expression in all conditions. 2% O₂ alone was superior in maintaining tenomodulin expression, while 21% O₂ culture required CM supplementation to do so. Importantly, transcript levels did not robustly correlate with protein levels indicating their potentially limited value as an output measure.

DISCUSSION & CONCLUSIONS: We have demonstrated that tendon-derived cell populations have distinct characteristics and that 2% O₂ was optimal for maintenance of tenogenic potential. The role of TSCs and their EVs in tendon repair presents an exciting and novel therapeutic opportunity.

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(2)[doi:10.1007/s00441-020-03379-3](https://doi.org/10.1007/s00441-020-03379-3)

(3)[doi:10.1089/ten.teb.2021.0075](https://doi.org/10.1089/ten.teb.2021.0075)

Keywords: Extracellular vesicles, Stem cell niche

PP-420

Comparative analysis of porcine adipose tissue-derived clonal populations reveal large heterogeneity in adipogenic capacity

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INTRODUCTION: A stromal vascular fraction (SVF) can be easily extracted from adipose tissue samples and contains relative high amounts of adipose-derived stem cells (ADSCs). SVF preparations are heterogenous and present variable adipogenic capacity in culture, depending among other factors on donor individual (1). Compared to humans and model species (2), relatively little knowledge exists on the nature of cell populations within SVF in farm species. A better understanding of such populations may allow the isolation and culture of bona fide adipogenic precursors for research or tissue engineering purposes (e.g. cultured meat) from these species. Therefore, the aim of this study was to isolate highly adipogenic clonal populations derived from SVF populations, as a base for further characterisation.

METHODS: Porcine adipose tissue was harvested from 1 week-old male piglets ($n = 4$) (1). SVFs were obtained and expanded in culture for 2-3 passages before single cell sorting via BD FACSAria Fusion. Individual clones were subsequently derived from each animal. Both parental and clonally-derived population were then cultured in adipogenic differentiation media, followed by quantitative Oil Red O analysis by spectrophotometry (510 nM) following 11 days differentiation (2). Selected clones were then analysed by mRNA sequencing in order to identify genetic markers associated with adipogenicity.

RESULTS: Around 150 clones were obtained from each animal, of which the 30 fastest growing ones were expanded further and analysed. Between 60% and 90% of all clones analysed were able to form adipocytes in culture as evidenced by both light microscopy imaging and quantitative Oil Red O (ORO) analysis. Adipogenic capacity was variable as evidenced by ORO intensities ranging between 0.1 and 0.08 within animal and 0.86 and 0.26 of most adipogenic clones between animals. In all animals, between 1 and 3 of the clonal populations outperformed the parental population in terms of differentiation capacity, as evidenced by ORO staining. Results of RNA-sequencing analyses will also be shown.

DISCUSSION & CONCLUSIONS: Individual clones were highly variable in terms of their adipogenic capacity in vitro. A majority of clones isolated from SVF preparations had reduced adipogenic capacity compared to parental population, suggesting that progenitors with the highest adipogenic potential may have a growth advantage within SVF preparations grown in culture. Ongoing RNA-sequencing analyses should provide valuable novel information on bona-fide adipogenic progenitor markers.

REFERENCES: Reference list intentionally left blank: required due to word count limit.

Keywords: Multipotent (mesenchymal) stem cells,

PP-421

Role of IGF axis in Bone Tissue Engineering using Diabetic BM-MSCs

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INTRODUCTION: Diabetes is a major health problem with a number of complications including diabetic osteopathy. Bone marrow mesenchymal stem cells (BM-MSCs) have been the subject of multiple clinical trials for bone tissue engineering. The insulin-like growth factor (IGF) axis plays a central role in stem cells proliferation and osteogenic differentiation, however the impact of diabetes on BM-MSCs osteogenic regenerative potentials and expression of IGF axis genes is yet to be established.

METHODS: BM-MSCs were isolated from knee joints of diabetic and non-diabetic donors (n=3). Colony-forming units fibroblasts (CFU-Fs) assay was used to evaluate their clonogenic potentials and MSCs surface markers and enumeration were assessed using multi-parameter flow cytometry. BM-MSCs were stained with alkaline phosphatase (ALP) stain and calcified deposits were stained and quantified using Alizarin Red quantitative assay. The gene expression of osteogenic markers *ALPL*, *RUNX2*, *OCN*, *OPG* and IGF axis *IGF-1*, *IGF-2*, *IGF1-R*, *IGF2-R* and *IGFBP-1 to 6* was evaluated using qPCR and the concentrations of IGFBP-2, 3 and 4 were measured in conditioned media using ELISA assays after 1, 2 and 3 weeks of culture under basal and osteogenic conditions.

RESULTS: There was no significant difference in the proportions of CFU-Fs ($9.58 \pm 2.42\%$ vs $10.07 \pm 6.41\%$), MSCs surface markers expression and MSCs population enumeration ($82.88 \pm 7.28\%$ vs $94.02 \pm 1.5\%$) in non-diabetic vs diabetic BM-MSCs respectively. Diabetic cells stained weakly for ALP but formed mineral deposits and expressed osteogenic markers comparably to non-diabetic cells. Diabetic BM-MSCs significantly expressed lower levels of *IGFBP-2*, *3* and *4* at Wk1, Wk3 and Wk1 of basal cultures respectively ($p < 0.05$) compared to non-diabetic cells. Levels of IGFBPs were similar in conditioned media of both cell populations but showed significant time-dependant increase ($p < 0.05$).

DISCUSSION & CONCLUSIONS: Diabetic BM-MSCs showed comparable stem cell characteristics and osteogenic differentiation potential to those of non-diabetic donors. However, they expressed lower levels of some IGFBPs genes which warrants confirming these findings in uncultured diabetic BM-MSCs. IGFBPs could be utilised as signalling cues for enhancing bone regeneration in health and under diabetic conditions.

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Keywords: Stem cells - general, Diabetic healing

PP-422

Development of an in vitro model of the bone marrow niche focussing on the mechanobiology of leukemic stem cells

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INTRODUCTION: The bone marrow niche contains a multitude of cell types and a microenvironment supportive of haematopoiesis. Mesenchymal stem cells (MSCs) secrete various growth factors which support the maintenance of haematopoietic stem cells (HSCs) in a quiescent state. Similarly, leukemic stem cells (LSCs) rely on these interactions in the bone marrow niche to support their survival and resistance to treatment. Improvements in the culturing of HSCs and MSCs in vitro have involved (1) the development of 3D culture models; (2) the co-culture of the two cell types; and (3) the addition of mechanical stimulation.

METHODS: Here, we have designed and optimised a system to recapitulate the 3D microenvironment of MSCs and HSCs by using synthetic fibronectin-based hydrogels in which the stiffness and degradability can be tuned. Fibronectin has a binding site for growth factors and therefore its incorporation into hydrogels allows us to mimic the role of the ECM to bind and sequester growth factors improving their bioavailability. Mechanical cues have an underappreciated role in fostering the correct functioning and survival of cells, therefore, the effect of applying mechanical stimulation to the model niche was explored by applying vibrations of nanoscale amplitude and measuring the bulk and local mechanical properties of the hydrogels.

RESULTS: We determined the optimal conditions to successfully develop hydrogels that mimic the bone marrow niche and showed that MSCs, HSCs and LSCs were viable when cultured in this 3D model. Further, we demonstrated that HSCs and LSCs respond differently to mechanical stimulation.

DISCUSSION & CONCLUSIONS: In conclusion, we have developed a method for the 3D culture of the bone marrow niche incorporating both HSCs and MSCs which can be utilised to investigate the mechanical changes in cell and ECM stiffness in response to malignant cells.

Keywords: Cancer, Biomechanics / biophysical stimuli and mechanotransduction

PP-423

Effects of Cytokines (TNF-ALPHA, IFN-GAMMA and IL-1BETA) on Equine Induced Pluripotent Stem Cells and Derivatives: activation of inflammatory pathways and change in gene expression

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INTRODUCTION: Musculoskeletal injuries (MKIs) are associated with morbidity and affect many people worldwide. MKIs have large economic and social impact. Trauma evokes a local and subsequent systemic inflammatory reaction to repair and regenerate the damage. When tissue integrity is restored, inflammation resolves. However, uncontrolled, or persistent inflammation appears to have a damaging effect on tissues. It has been demonstrated that embryonic stem cells (ESCs) and their derivatives are resistant to inflammation which may be advantageous in their clinical application. However, it is unclear if induced pluripotent stem cells (iPSC) and their differentiated progeny share this property. The anatomy and biology of horses lend equine model systems as an attractive animal model of human musculoskeletal injuries. This project aims to evaluate whether equine iPSCs and their differentiated progeny are responsive to pro-inflammatory cytokines

METHODS: We cultured undifferentiated equine iPSCs, iPSC-derived tendon-like cells, iPSC-derived cartilage-like cells and iPSC that have undergone spontaneous differentiation into a range of cell types in presence or absence of Interferon gamma (IFN γ), Tumor necrosis factor (TNF α) and Interleukin-1beta (IL-1 β). Localization of Nuclear factor kappa subunit 1 (NF κ B), c-Jun N-terminal kinase 1/2/3 (JNK1/2/3), Signal transducer and activator of transcription 1 (STAT1) and p38 mitogen-activated protein kinases (p38 MAPK) was analysed by immunofluorescence after 1 hour stimulation. We used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to analyse the effect of the three cytokines on the expression of genes including matrix metalloproteases (MMPs), cell type-associated genes and inflammatory-associated genes after 72 hours stimulation.

RESULTS: Immunofluorescence analysis of NF κ B, JNK1/2/3, STAT1 and p38 MAPK revealed that iPSCs and their derivatives do respond to cytokines, with equine iPSC-tenocytes cells producing the same NF κ B signalling response as primary equine tendon cells. Microscope imaging and gene expression showed a negative effect of the three cytokines on undifferentiated cells. We observed significant changes on the expression of cell type-associated genes. In the case of iPSC-derived tendon cells, *MMP3* and *MMP13* genes were upregulated after cytokine exposure, as is observed in primary tendon cells. In general, we observed an increase in the expression of inflammatory genes *IL6*, *IFNG*, *STAT3*, *TNF* and *IL1B* in each cell type.

DISCUSSION & CONCLUSIONS: In conclusion, treatment with IFN γ , TNF α and IL-1 β triggers a response in equine iPSCs and their derivatives. The results inform the development of future stem cell-based treatments and will complement ongoing work to develop novel drug treatments that can protect the musculoskeletal system from inflammation to enable better tissue regeneration.

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Differentiation

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IN VITRO ASSESSMENT OF THE THERAPEUTIC POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STROMAL CELLS (iMSCs)

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INTRODUCTION: Human Mesenchymal stromal cells (MSCs) represent a novel treatment for OA. A key mechanism of action of MSCs is their ability to modulate the immune system. MSCs can respond to the pro-inflammatory signals in their implanted environment by altering the surrounding immune cells by secreting anti-inflammatory signals through cell-to-cell contact as well as undergoing apoptosis. In vitro licensing of MSCs with pro-inflammatory cytokines demonstrates an increased anti-inflammatory response and licensed MSCs may offer a more therapeutically effective treatment option. iMSCs may also be an alternative cell source for MSCs. However, little is known about their ability to immunomodulate. This work aimed to establish the immunomodulatory capacity of iMSCs licensed with inflammatory factors relevant to the OA environment.

METHODS: Human MSCs/iMSCs were exposed to six pro-inflammatory factors, and the response in the expression of six anti-inflammatory genes was assessed using qPCR. The effects of MSC/iMSCs and tumour necrosis Factor (TNF- α) and interferon (IFN- γ) on licensed MSC/iMSCs was then investigated in T-cell and monocyte co-cultures. To determine the role of licensing on MSC/iMSCs apoptosis, MSCs and iMSCs were licensed with the same six factors and apoptosis was induced by exposure to staurosporine (STS).

RESULTS: Data demonstrated that iMSCs treated with OA-relevant factors share a similar anti-inflammatory secretome to MSCs. qPCR results show that licensing MSCs/iMSCs with TNF- α and IFN- γ at 50ng/ml increased the expression of IDO, TSG-6, PTSG-2, and IL-6. In a T-cell co-culture system, iMSCs were shown to significantly reduce the proliferation of T-cells ($p < 0.05$). MSCs and iMSCs licensed with TNF- α and IFN- γ were also cultured with T-cells but they failed to significantly reduce T-cell activation. Similarly, the effect of MSCs/iMSCs on LPS-stimulated monocytes was investigated. MSCs/iMSCs reduced the expression of the pro-inflammatory cytokine TNF- α in monocytes ($p < 0.05$). Lastly, licensed MSCs/iMSC had an accelerated response to the STS treatment.

DISCUSSION & CONCLUSIONS: This study demonstrates that iMSCs may be a viable alternative source of MSC. iMSCs shared a comparable anti-inflammatory secretome after licensing to primary MSCs and iMSCs equalled MSCs in their ability to significantly reduce T-cell and monocyte activation. TNF- α and IFN- γ licensed MSCs/iMSCs behaved similarly in their response to STS treatment, indicating increased susceptibility to apoptosis. iMSCs share similar characteristics to primary MSCs, highlighting the option of iMSCs as a viable alternative cell source for future OA treatments.

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Keywords: Induced pluripotent stem cells, Immunity / immunomodulation / macrophage

PP-425

TNF-alpha and SIRT1 modulation affects bioenergetics and chondrogenic capacity of mesenchymal stromal cells

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Erasmus MC

INTRODUCTION: Mesenchymal stromal cells (MSCs) are promising for cell-based cartilage tissue engineering strategies as they can differentiate into the chondrogenic lineage. Although the inflammatory environment often present in the diseased joint can negatively influence MSC chondrogenic differentiation and cartilage maintenance, inflammation may also have a positive role. In fact, we previously demonstrated that the inflammatory factor TNF α enhanced chondrogenesis of MSC when added prior differentiation. Interestingly, the master regulator of cellular metabolism sirtuin1 (SIRT1) has also been reported to enhance chondrogenesis in MSC, but it can be inhibited by TNF- α . Here we aimed to investigate the combinatory effect of the TNF- α treatment with a SIRT1 activator, in order to evaluate the ability of this co-treatment to further enhance chondrogenic differentiation of MSC.

METHODS: Human MSCs (N=5) were isolated and expanded in a 10% fetal calf serum-containing media supplemented with 1 ng/ml FGF-2 (basic media). After, cells were further expanded for one additional passage divided in 4 conditions consisting in the basic media supplemented with (1) vehicle; (2) 50 ng/ml TNF α ; (3) 1 μ M SRT1720, a SIRT1 activator; (4) 50 ng/ml TNF α + 1 μ M SRT1720. Concentration of TNF α was based on our previous publication, and concentration of SRT1720 was based on a screening evaluating specificity and toxicity of the compound. Chondrogenesis was induced by 3D pellet cultures using a TGF β -based media for 21 days, and it was evaluated by (immune)histochemistry (Collagen type-2 and thionine for glycosaminoglycans) and RT-PCR (AGN and COL2A1). Prior chondrogenesis, monolayer cells were evaluated for bioenergetics parameters (XF-Seahorse Analyzer, Bioscience) and β -catenin pathway activation (western blot).

RESULTS: After chondrogenic induction, TNF α -expanded cells resulted to be the most chondrogenic, while the TNF α + SRT1720-expanded cells were the least chondrogenic, often completely negative for Collagen type-2 staining. Interestingly, an in-depth bioenergetics analysis revealed that the MSC stimulated with TNF α + SRT1720 had the higher levels of basal and maximal oxygen consumption rate (OCR), and of the extracellular acidification rate (ECAR), indicating an overall metabolic enhancement. Analysis of the WNT/ β -catenin signaling on those cells, confirmed our previous observation that TNF α -stimulated cells have enhanced β -catenin activity, but also showed that further administration of SRT1720 inhibited β -catenin activity.

DISCUSSION & CONCLUSIONS: Co-stimulation with TNF α and the SIRT1 activator SRT1720 during expansion is detrimental for chondrogenic capacity of MSC. This is possibly due to an hyper-metabolic activation in these cells and a negative effect on the WNT signaling pathway, parameters known to sustain MSC differentiation.

Keywords: Cartilage / joint and arthritic conditions, Stem cells - general

PP-426

Direct and indirect effects of regulatory T cells on the osteogenic potential of mesenchymal stromal cells

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INTRODUCTION: Bone marrow stromal cells (BMSC) are widely used in bone regeneration due to their multipotency potential. Regulatory T cells (Treg) have shown to play a role in bone repair, but if Treg controls the fate of BMSC into the osteogenic lineage is still not completely understood. Therefore, the scope of this study was to investigate the impact of Treg on the osteogenic potential of BMSC through direct or indirect interactions.

METHODS: For direct interaction, BMSC were co-cultured with Treg (1:3) under routine growth media for 14 days. For indirect co-culture, BMSC were cultured for 14 days in 10% or 50% condition media from Treg (Treg-CM). The morphological changes were followed by phase contrast microscopy. Furthermore, differentiation of BMSC were assessed at gene level by RT-PCR and by alkaline phosphatase enzyme activity assay in both interaction conditions.

RESULTS: Our results revealed that BMSC morphology changed from fibroblast-like to a more spindle elongated shape when cultured with Treg or with Treg-CM. Early osteogenic markers, RUNX2 and Col1A2, were downregulated when BMSC were co-cultured with Treg or Treg-CM. Interestingly, expression of late osteogenic marker, SPP1, was upregulated in BMSC cultured with Treg or Treg-CM compared to cells cultured only in routine growth media. Furthermore, ALP enzyme activity of BMSC was increased significantly with 10% and 50% Treg-CM compared to cells cultured in growth media alone.

DISCUSSION & CONCLUSIONS: Cellular differentiation is a shift from proliferation to specialization and involves morphological and molecular changes. The morphological changes observed might indicate the cells become confluent earlier due to rapid proliferation with Treg or Treg-CM addition, which may have resulted in earlier differentiation. To confirm this, we checked the expression of osteogenic related genes, and noticed that while the expressions of early osteogenic markers were downregulated, SPP1 expression was upregulated in cells which cultured with Treg and Treg-CM compared to BMSC cultured with growth media alone. While the ALP enzymatic activity was significantly increased in BMSC when treated with Treg-CM, there was no significant difference in direct coculture. This might indicate the secretome from Treg has more impact on BMSC osteogenic differentiation rather than a direct cellular interaction. In conclusion, our findings suggests that Treg and Treg secretome have a promising potential to promote osteogenic differentiation of BMSCs. Dissecting the exact molecular mechanisms behind BMSC-Treg crosstalk can improve the efficiency of BMSC-based bone regeneration therapies.

ACKNOWLEDGEMENTS: This work was funded by Trond Mohn Foundation (TMS2021STG03) and Norwegian Research Council (314473)

Keywords: Bone and bone disorders (osteoporosis etc), Immunity / immunomodulation / macrophage

PP-427

Mesenchymal-like cells isolated from menstrual blood, comparing sanitary pads, tampons, and menstrual cups for accessible donation

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INTRODUCTION: Menstrual blood (MB) contains mesenchymal-like cells (MenSC) which are highly proliferative, capable of multilineage differentiation, with strong migratory capacity and immunomodulatory abilities. The cyclicity and non-invasive nature of MB donation makes MenSC a promising candidate for regenerative medicine. A common donation method is via a menstrual cup (MC). However, only 4% of the UK currently use one, with most preferring tampons and sanitary pads (SP). Therefore, this study aimed to identify MenSC isolation methods from SP and tampons to extend the accessibility of MenSC research and future clinical applications.

METHODS: In this ongoing study, healthy women aged between 21-41 (n = 19) donated MB samples (n = 31) on their heaviest flow day, using a SP (n = 14), tampon (n = 10), or MC (n = 7). Isolation success rate and metabolic activity is assessed via 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, an indicator of cell metabolism and therefore viability. Stemness is confirmed using antibodies and their detection by flow cytometry; trilineage differentiation potential is confirmed using fat, bone and cartilage differentiation. Cell viability, immediate versus delayed (48 h) processing, and direct versus fractionating prior to culture is evaluated using MTT.

RESULTS: MenSC were isolated and cultured from all three products; SP, tampon, and MC for the first time, including immediate and delayed processing. Tampons and MC had a greater MenSC isolation success rate (immediate processing: SP = 38%, tampon = 83%, MC = 35% success rate; delayed processing: SP = 33%, tampon = 75%, MC = 75% success rate), but SP are the most popular MB donation method (S P= 75%, tampon = 60%, and MC = 35% willingness rate). MenSC displayed high proliferative capacity, a doubling time between 21-89 h. Confirmation of stemness and trilineage potential are currently ongoing. Participants described the donation as easy and empowering.

DISCUSSION & CONCLUSIONS: This research is the first comparison of MB donation techniques, comparing SP, tampons, and MC. Although tampons and MC had a greater MenSC isolation success rate, SP are the more popular method among participants and the population, therefore there is value in using all three methods. The ability to temporarily store MB samples at 4°C makes MB donation practical. MenSC offer unique advantages including non-invasive, cyclical collection, and are a promising source of cells for regenerative therapies in the future. The ability to donate MB with SP and tampons in addition to MC makes this approach to cell donation even more accessible.

Keywords: Stem cells - general,

PP-428

Total Autologous Angiogenic Therapy with Cultured Bone Marrow Derived Mesenchymal Stromal Cells Grown in Platelet-Rich Plasma Against Peripheral Arterial Disease on Japanese Advanced Medical Care

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INTRODUCTION: Previous clinical studies have reported that the implantation of bone marrow (BM)-derived mononuclear cells (MNCs), crude mesenchymal stem cells (MSCs) or MSCs cultured with fetal bovine serum results in improvement in symptoms and healing of ulcers in patients with critical limb ischemia (CLI). However, some problems related with the efficacy and safety remained in those therapies. We have started a multicenter prospective interventional trial with cultured bone marrow derived MSCs grown in platelet-rich plasma (PRP) against CLI on Japanese Advanced Medical Care which the cost of treatment is paid by patient and also Japanese national insurance. (Number of Japanese Advanced Medical Care 57)

We present the protocol of our new trial using angiogenesis with total autologous angiogenic therapy.

METHODS: **AIM:** To assess efficacy of our regenerative therapy with autologous cultured bone marrow derived MSCs grown in autologous platelet-rich plasma (PRP) against advanced chronic critical limb ischemia in patient with diabetes and renal dysfunction.

Design: Multicenter, single arm, prospective interventional study.

Patients and METHODS: Fifty patients with CLI will be enrolled to this trial. The ethics committee of Tokyo Medical University Hospital and the Evaluation Committee on Regenerative Medicine approved this study and informed consent was obtained from the patient. Trial was started from April 2022 for five years. Twenty ml of bone marrow were aspirated from the iliac bones of the patient under local anesthesia. It was cultured with autologous PRP to more than 3×10^7 cells. The BM-MSC concentrate was suspended to more than 25 ml with sterile saline. Under venous anesthesia, 0.5 ml of that was injected to muscle in ischemic lower limb. Primary end point was rate of limb salvage 1 year after cell therapy. And following examinations were done as ulcer size if it existed, 6 minutes walking test, visual analogue scale (VAS), skin perfusion pressure (SPP), trans cutaneous oxygen tension (TcPO₂) and ankle brachial pressure index (ABPI).

RESULTS: Our trial is going now, we can't show final results.

DISCUSSION & CONCLUSIONS: We could present some results of limited number patients undergone cell therapy on meeting day.

Keywords: Multipotent (mesenchymal) stem cells, Vascular systems / vascularisation and heart

PP-429

3D collagen-embedded spheroids of periodontal ligament stem cells enhance cementogenic differentiation via plasminogen activator inhibitor 1

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INTRODUCTION: A recent study suggested that plasminogen activator-inhibitor type 1 (PAI-1) can induce the cementogenic differentiation in the periodontal ligament stem cells. Embedding spheroids within a hydrogel promotes cellular differentiation and cell–matrix attachment, making spheroids appropriate models of human periodontal ligament stem cells (HPLSC) microenvironment. Thus, the present study examined the effects of recombinant human PAI-1 (rhPAI-1) treatment on enhanced cementogenesis in collagen-embedded HPLSC spheroids.

METHODS: We cultured HPLSCs as spheroids that were embedded in collagen gel to examine whether their cementogenic differentiation could be enhanced by treatment with rhPAI-1.

RESULTS: The upregulated expression of cementum protein 1 (CEMP1) and cementum attachment protein (CAP), established cementoblast markers, was observed in the 2D monolayer HPLSCs that were treated with rhPAI-1 for 3 weeks compared with that in the control and osteogenicinduction medium (OIM) groups. In the embedded HPLSC spheroids, rhPAI-1 treatment induced interplay between the spheroids and collagenous extracellular matrix (ECM), indicating that disaggregated HPLSCs migrated and spread into the surrounding ECM 72 h after three-dimensional (3D) culture. Western blot and immunocytochemistry analyses showed that the CEMP1 expression levels were significantly upregulated in the rhPAI-1-treated embedded HPLSC spheroids compared with all the 2D monolayer HPLSCs groups and the 3D spheroid groups.

DISCUSSION & CONCLUSIONS: Our findings suggest that rhPAI-1 promotes cementoblastic differentiation in 2D monolayer HPLSCs. Treatment with rhPAI-1 showed disaggregation of the embedded HPLSC spheroids in the early stages compared with the control and OIM-treated groups. Our findings concerning the early disaggregation of rhPAI-1-treated spheroids may indicate that, in addition to the migratory cells that were localized at periphery portion of the spheroids, the cells that were involved in the nonmigratory phase also changed to migratory cells. Therefore, rhPAI-1 treatment may enable the interplay of embedded spheroids and collagen in the ECM, which contributes to the induction of cementogenic differentiation of HPLSC spheroids. In our Western blotting and immunocytochemistry findings, CEMP1 expression was significantly upregulated in the embedded spheroids that were treated with rhPAI-1 compared with all groups in the 2D culture and the control and the OIM-treated spheroid-treated groups. These findings indicate that PAI-1-treated spheroids show a greater cementogenic differentiation capacity than the other groups.

Keywords: Differentiation,

PP-430

Tailored 3D Tendon Biomimetic Scaffolds Enhance Amniotic Epithelial Stem Cells Tenogenic Paracrine Regenerative Potential

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INTRODUCTION: Amniotic epithelial stem cells (AECs) represent an attractive source for tendon regeneration since they are able to undergo a stepwise epithelial-mesenchymal transition (EMT) and subsequent teno-differentiation both *in vitro* and *in vivo* [1,2]. This stepwise process is boosted in AECs engineered on tendon biomimetic PLGA 3D scaffolds with highly aligned fibers (AECs-3DS) [3]. Thus, this work aimed to evaluate AECs-3DS's paracrine potential by analyzing tenogenic molecules release in their conditioned media (CM) and its biological effect on tendon organoids' formation.

METHODS: Molecules secretion was assessed in CM derived from AECs, AECs cultured on PLGA fleeces (AECs-2D), and AECs-3DS after 48h of culture through a protein antibody array analysis (Abcam). CM tenogenic biological effect was evaluated in a validated *in vitro* indirect trans-well co-culture system [2,4] of AECs with AECs-2D or AECs-3DS for 14d. Tendon organoids' characterization was assessed by analyzing their morphology and TNMD, a late tendon related marker, protein expression (immunofluorescence and Western Blot).

RESULTS: The analysis of CMs, obtained from AECs cultured under different conditions, revealed a modulation of their secretion profile. In particular, AECs-3DS-CM showed higher levels of tenogenic related proteins such as RANTES, PDGF-BB, and b-FGF, compared to AECs and AECs-2D ($p < 0.05$).

Moreover, AECs-3DS' secreted molecules improved tendon organoids' formation leading to a significant higher average number of tendon-like structures, with a higher width and length with respect to AECs-2D. Tendon organoids' formation was confirmed by the phenotypic morphological change of AECs, which acquired a tenocyte-like shape, and the localization and a higher expression of TNMD protein when co-cultured with AECs-3DS with respect to AECs-2D ($p < 0.05$).

DISCUSSION & CONCLUSIONS: The teno-differentiated cells, cultured within both constructs, released molecules known to be involved in tendon healing. In particular, AECs-3DS-CM showed a more instructive tenogenic biological effect probably due to the 3D milieu that better mimics the tenogenic microenvironment. In this condition, in fact, the release of key tenogenic molecules was boosted and able to induce a more efficacious tendon organoids' formation. Further investigations are needed to characterize the obtained organoids that could be used to study mechanisms of disease and tools applicable for drug testing. Contextually AECs-3DS stand as potential candidates for tendon regeneration and as an innovative system to produce tenogenic CM.

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Keywords: In vitro microenvironments, Differentiation

PP-431

Tailoring the microporosity of embroidered structures by melt electrowriting to enhance the cellular alignment on hierarchical tendon and ligament replacements

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INTRODUCTION: The additive manufacturing (AM) technique melt electrowriting (MEW) allows the fabrication of highly defined fibre scaffolds with structural features within the dimension of cells, which can be used to tailor the scaffold-cell interaction towards an oriented cellular growth. On their own, these scaffolds lack the mechanical properties to mimic tendons and ligaments. However, embroidered structures closely imitate these characteristics but their macroporosity aggravates a defined cell interaction, which is crucial for a tendon/ligament-like cell alignment and a differentiation of migrating stem cells. Here, we present a novel combination of both AM-techniques leading to scaffolds with a highly defined micro- and macroporosity. Static culturing of human adipose-derived mesenchymal stem cells (AT-MSC) on the composites showed a strongly oriented cell growth.

METHODS: MEW was executed with a GeSiM BioScaffolder 3.1. combined with a MEW print head (GeSiM mbH, Germany) by melting medical grade polycaprolactone (PCL, Corbion, Netherlands) to 73-85°C. For the embroidery process (TLMX-901, Tajima Ind., Germany) a monofilament suture thread made of poly(lactic-co-ε-caprolactone) (P(LA-CL), USP 7-0, Gunze Ltd., Japan) was used. Scaffolds were composed of one plie with a zigzag-pattern design with an additional line reinforcement applied horizontally. Medical grade collagen type I isolated from rat-tail (Meidrix Biomedicals GmbH, Germany) was implemented as additional coating. AT-MSC were cultivated on scaffolds for 14 days.

RESULTS: As multiple PCL-fibre arrangements were used to achieve oriented cell growth in literature, a comparison was conducted to explore the highest cell alignment depending on the scaffold geometry. Therefore, fibre diameters of 5 and 15 µm, different pore geometries with angles of 90°/90°, 30°/160° or 70°/110° and different fibre spacing (100-250µm) were evaluated. The strongest oriented cell growth was obtained with a fibre diameter of 5µm, angles of 30°/160° and a fibre spacing of 150µm. Subsequently, the addition of the collagen coating increased the cell-seeding efficiency and accelerated the cell coverage of scaffolds while maintaining a clear oriented cellular growth. Adapting the MEW-parameters to the inhomogeneity of the otherwise homogenous electrical field raised by the presence of the embroidery structures resulted in a microporosity similar to the programmed G-Code while maintaining a continuous fibre diameter. AT-MSC showed a higher oriented cellular growth on embroidered structures with a MEW-mesh than without. Furthermore, additional mechanical stimulation suggest a stronger differentiation of AT-MSC towards tenocytes through the implementation of the microporosity.

DISCUSSION & CONCLUSIONS: The high biocompatibility of this combined approach together with the cell alignment were innovative steps towards novel solutions for tendon/ligament replacements.

Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Additive manufacturing

PP-432

Development of *in vitro* muscle tendon junction using electrospun scaffold

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INTRODUCTION: The muscle tendon junction (MTJ) transmits force generated by the muscle to the tendon and ultimately to the bone, and is associated with muscle strains and tears. When a tear occurs at the MTJ, its regeneration is limited due to poor vascularisation, and there is no cure for a complete tear. Therefore, the development of a tissue engineered MTJ would be beneficial. This project aims to develop a 3D MTJ *in vitro* using electrospun fibres.

METHODS: Human myoblasts and tenocytes were co-cultured on various substrates to determine the factors for the development of MTJ. Conditions included: 2D culture on plastic; 3D culture on either randomly deposited fibres or aligned fibres; pure polycaprolactone (PCL) fibres or fibres of PCL and decellularized extracellular matrix (DECM) from either muscle or tendon. All fibres were obtained by electrospinning, and the aligned fibres were produced using a gap collector. Cell morphology was assessed using immunocytochemistry to analyse the formation of junction's specific structure (finger-like processes) and to detect the expression of collagen 22 for the evaluation of the most appropriate environment for the development of an *in vitro* MTJ. Furthermore, the effect of DECM on myogenic and tenogenic differentiation was investigated using human mesenchymal stem cells (MSCs). Myogenic and tenogenic differentiation was assessed using antibodies directed towards desmin and tenomodulin, respectively.

RESULTS: Human myoblasts and tenocytes expressed collagen 22 both in 2D and 3D co-culture, with a higher expression observed when cultured in 3D; whereas the mono-culture of human myoblasts and tenocytes did not show collagen 22 expression in either culturing conditions. Aligned fibres promoted cell alignment, mimicking the structure of native MTJ, however, finger-like processes similar to the morphology of a natural MTJ were not observed. Although DECM fibres did not seem to have an effect on collagen 22 expression compared to PCL fibres (control), they induced MSCs to differentiate into myoblasts and tenocytes.

DISCUSSION & CONCLUSIONS: Co-culture of myoblasts and tenocytes in a 3D environment was successfully performed. Culturing both myoblasts and tenocytes together in a 3D environment was crucial for the formation of the MTJ, and aligned fibres induced a more discernible interface, however even in 3D, finger-like processes were not observed. Data showed that DECM can induce myogenic and tenogenic differentiation. Further investigation is needed such as qPCR to quantify differentiation of MSCs into tenocytes or myoblasts, and the use of a bioreactor to apply cyclic load to induce and mature MTJ formation.

Keywords: Biomaterials, Musculoskeletal (inc ligament / tendon / muscle / etc)

PP-433

IVC/DVC-2 digital cage to predict tendon structural recovery

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INTRODUCTION: IVC Digital-Ventilated-Cages (IVC/DVC-2) are an innovative type of cages that allow to obtain accurate movement data from a single mouse over time [1]. Thus, they could be used to determine the occurrence of a tendon damage event as well as inform on tissue regeneration [2,3]. Therefore, using the mouse model of tendon experimental damage, in this study it has been tested whether the recovery of tissue microarchitecture and of extracellular matrix (ECM) correlates with the motion data collected through this technology.

METHODS: According to animal experimentation authorised by the Italian Ministry of Health (183/2021), mice models were used to induce acute injury in Achilles tendons (ATs), while healthy ones were used as control. During the healing process, the mice were housed in IVC/DVC-2 type cages (Tecniplast), that were used to monitor animal welfare and to study biomechanics assessing movement activity, an indicator of the recovery of tendon tissue functionality. After 28 days, the ATs were harvested and assessed for their histology (Hematoxylin-Eosin and Alizarine-Red), and collagen type 1 (COL1) protein expression by immunofluorescence (IF) assays and correlated to movement data.

RESULTS: Healthy mice tendons, used as control, were compared to injured ones to observe the structural changes that occur during spontaneous tendon healing. Hypocellularity, organized ECM, and parallel COL1 fibres are characteristics of healthy tendon tissue. Differently, during spontaneous healing, a disorganized tissue with hypercellularity was observed within the lesioned tendons together with the presence of calcified foci (confirmed by Alizarine-Red staining) around the many chondrocytes and throughout the lesioned areas. IF assays showed a disorganization of COL1 deposition in the injured tendon tissue compared to the control. Movement data is currently being analysed to predict a possible correlation between functional recovery and histological data.

DISCUSSION & CONCLUSIONS: The preliminary results showed structural and movement differences between healthy and spontaneously healed tendons. Most of the injured tendons showed chondrocyte-like cells, various calcification stages, and major structural changes, which may suggest that spontaneous healing does not provide a complete tendon healing and thus, a complete recovery of the biomechanics. These findings will be confirmed by examining the IVC/DVC-2 big data analyses that will indicate the progression of tendon healing up to complete recovery of movement activity after spontaneous tendon healing.

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Keywords: Veterinary, In vivo and animal models

PP-434

Optimisation of a spheroid based approach for vascularisation of primary 3D tissue engineered skeletal muscle constructs

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INTRODUCTION: Our lab has developed robust and reproducible tissue-engineered 3D skeletal muscle (SkM) models that have been used to study the physiological and genetic mechanisms initiated in response to skeletal muscle injury and subsequent regeneration.

To increase the physiological relevance of these models, we plan to introduce a vasculature (using a spheroid-based approach) to differentiated SkM tissues and investigate whether this impacts the structure, baseline function and injury response and recovery of the SkM.

We postulate that spheroids seeded onto (or into) SkM cultures will sprout networks and infiltrate the SkM forming a vasculature, a method successfully used to generate vasculature in cardiomyocytes (1).

METHODS: A mixture of HDFs and HUVEC-GFP to a total of 5000 cells were seeded into non-adherent plates in EGM-2 media to generate spheroids. The vasculogenic potential of spheroids with HDFs:HUVEC-GFP ratios of 4:1, 1:1, 1:4, 1:0, 0:1, as well as spheroid culture time were optimised. Spheroids were genetically analysed or plated onto 75% collagen/25% Matrigel in EGM-2 media and imaged by fluorescence microscopy at 1 day time intervals for 7 days. Vasculogenesis was quantified by measuring cumulative sprout length, sprout number and number of junctions and by quantification of CD31, angiopoietin1 and angiopoietin 2 gene expression.

Optimal collagen/Matrigel post-seeding time-point was also determined. 4:1 HDF:HUVEC-GFP ratio spheroids cultured for 24 hours were seeded onto 75% collagen/25% Matrigel. Network formation was quantified via immunocytochemistry and genetic analysis of CD31, angiopoietin1 and angiopoietin 2 expression at days 1, 3, 5, 7 and 14, as described above.

RESULTS: Spheroids cultured for 24 and 48 hours with 4:1 ratio of HDF:HUVEC-GFP had twice and four times the cumulative sprout length and number than 1:1 or 1:4 ratio spheroids, respectively. The HUVEC-GFP only spheroids formed no network. No difference in vasculogenesis was observed between the spheroid ratios tested from 72 hours onwards.

Spheroids cultured with 4:1 and 1:1 ratios of HDF:HUVEC-GFP for 24 and 48 hours were equally vasculogenic, but by 72 hours this network formation potential dropped by half and by half again by 5 days. Vasculogenic potential of 1:4 ratio cells did not change over culture time-course but was significantly lower throughout all time-points.

DISCUSSION & CONCLUSIONS: We conclude that HDFs are required to optimise HUVEC network formation when cultured as spheroids. Spheroids cultured for 24 or 48 hours with a HDF:HUVEC-GFP ratio of 4:1 will likely have the most vasculogenic potential when seeded onto skeletal muscle.

REFERENCES: Adv Sci (Weinh) 2022 Mar;9(9):e2104299, liu et al

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Vascular systems / vascularisation and heart

PP-435

Xeno-free bioengineered human skeletal muscle tissues

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INTRODUCTION: Bioengineered human skeletal muscle tissues have emerged in the last years as new *in vitro* systems for disease modelling. These bioartificial muscles are usually fabricated by encapsulating human myogenic precursor cells in a hydrogel scaffold that resembles the extracellular matrix (ECM). Nowadays, most of these hydrogels are derived from xenogenic sources, such as gelatin from porcine skin, collagen from rat tail, fibrinogen from bovine plasma, or decellularized ECM from porcine muscle. This limits any clinical application of engineered tissues in regenerative medicine due to the possible presence of xenogenic contaminants from animal-derived ECM.

Moreover, animal-derived serum in culture media could reduce sensitivity to drug toxicity within *in vitro* testing platforms. These limitations can be overcome by using xeno-free biomaterials and culture conditions, which offer increased relevance for developing human disease models.

METHODS: In this work, for the first time, human platelet lysate-based nanocomposite hydrogels (HUGel) were used as scaffolds for human skeletal muscle tissue engineering in a xeno-free culture. These hydrogels consist of human platelet lysate reinforced with cellulose nanocrystals that allow tunable mechanical, structural, and biochemical properties for the 3D culture of stem cells. Here, we fabricated hydrogel casting platforms for the encapsulation of human muscle satellite stem cells in HUGel around a pair of flexible posts, which act as tendon-like anchoring sites that aid cell alignment and myotube formation. Furthermore, the content of cellulose nanocrystals was modulated to obtain long-lasting scaffolds with high clot retraction properties that promote skeletal muscle tissue formation.

RESULTS: We demonstrated that this optimization enhanced matrix remodelling, uniaxial tension, and self-organization of the cells, resulting in the formation of highly aligned, long myotubes expressing sarcomeric proteins. The xeno-free bioengineered human muscles were subjected to a frequency sweep electrical pulse stimulation regime. The electrically stimulated tissues presented twitch or tetanic contractions depending on the applied frequencies. Moreover, the exerted contractile forces were measured in a non-invasive manner.

DISCUSSION & CONCLUSIONS: Overall, our results demonstrated that bioengineered human skeletal muscles could be built in xeno-free cell culture platforms to assess tissue functionality. These tissues could be used as patient-specific models for muscular diseases by incorporating patient-derived platelet lysate and satellite cells. Remarkably, the xeno-free characteristics of this *in vitro* 3D model could also enable the transition into clinical applications for regenerative medicine.

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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Hydrogels and injectable systems

PP-436

Peripheral blood mononuclear cell contribution to myogenic commitment of human Mesenchymal Stem Cells in a 2D and 3D Myoblast co-culture

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INTRODUCTION: The development of in-vitro skeletal muscle regeneration models is extremely challenging. However, bone marrow-derived Mesenchymal Stem Cells (BM-MSCs) can successfully differentiate toward a myogenic phenotype when co-cultured with skeletal Myoblasts (SkMs) in the presence of b-Fibroblast Growth Factor (b-FGF) at 10 ng/mL [1-2]. Contribution of peripheral blood mononuclear cells (PBMCs) in myogenesis is still under debate [3]. Therefore, PBMCs were added to well-established 2D and 3D in vitro myogenic models composed by a co-culture of BM-MSCs and SkMs to investigate their potential contribution to BM-MSC myogenic commitment.

METHODS: PBMCs were added in an upper transwell chamber in standard 2D condition to investigate their paracrine effects on BM-MSC-SkM co-culture. Conversely, 3D scaffold-based systems were assembled using fibrin hydrogel mixed with a solution containing BM-MSCs, SkMs, and PBMCs. Scaffolds were cultured in perfusion conditions to study paracrine and cell-to-cell contact influence of PBMCs on myogenesis.

RESULTS: In 2D conditions, BM-MSC commitment towards a myogenic phenotype was confirmed by qRT-PCR showing upregulation of myogenic-related genes, such as Pax3, MyoD1, Myf5, Myf6, Desmin and Myosin Heavy Chain II (MYH2). In particular, Desmin and MYH2 expression, at mRNA and protein levels, was higher in the presence of PBMCs than that without peripheral cells, suggesting a synergist paracrine effect on myogenic commitment. In 3D conditions, myogenic markers were upregulated regardless the presence of PBMCs, while Desmin expression at both mRNA and protein levels seemed to be induced by the presence of PBMCs. In both co-culture systems, pro-inflammatory cytokine levels at both mRNA and protein levels decreased, while anti-inflammatory cytokines increased.

DISCUSSION & CONCLUSIONS: Our results support the contribution of PBMCs to BM-MSC myogenic commitment, and that 3D environment in dynamic culture could represent an extremely favorable biomimetic environment allowing the study of all myogenic marker expression at both gene and protein levels.

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Keywords: Hydrogels and injectable systems, Bioreactors

PP-437

Regenerative Programming of Animal and Human Stem Cells for Engineered Skeletal Muscle

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INTRODUCTION: Skeletal muscle is the most abundant tissue in the human body accounting for 30-45% of total body mass, generating forces that allow for the movement of limbs and locomotion. After a minor injury, muscle has the capability of regeneration, however more traumatic injuries can bring about permanent limitations of functional movement.

The current treatment performed is the autologous transplant of muscle tissue from distant or local sites to the site of injury. However, research indicates that therapies that combine biomaterial scaffolds and cell therapies achieve the greatest improvement in muscle function after injury. This project aims to repurpose blood cells and blood constituents to make a 3D tissue-engineered muscle structure intended for autologous tissue transplant.

METHODS: Peripheral blood mononuclear cells (PBMCs) are extracted from either whole blood or buffy coat provided by NHS Blood and Transplant. These cells are transfected via glycosaminoglycan (GAG) binding enhanced transduction (GET) non-viral gene delivery to deliver myogenic factors like MyoD and Pax7 to direct the cells to a myogenic lineage. These cells are then used in conjunction with peptide amphiphile (PA) gels in attempts to construct a patient specific skeletal muscle structure. These PA gels can also be mixed with other compounds such as plasma and serum in attempts to enhance cell adhesion and viability in the biomaterial scaffold.

RESULTS: Native PBMCs that were unstimulated poorly express transfected genes. However, after Phytohaemagglutinin (PHA) activation GET transfected genes express more efficiently. Gene products were expressed maximally when transfection occurred during activation (0 Days) and 7 days after activation. 5-, 3- and 1-day(s) PHA stimulation prior to transfection show lower or no transfection in comparison. Light microscopy pictures show that 7 days of stimulation prior to transfection show a change in their morphology and these differentiated cells seem to be responsible for the higher transfection efficiency.

DISCUSSION & CONCLUSIONS: The project has established that we can transfect the notoriously difficult to transfect PBMCs with the non-viral GET system. This is with the aid of PHA, which triggers the activation and subsequent proliferation of PBMCs. Working theory is that upon proliferation the nuclear envelope opens thus allowing the GET nanoparticle to enter the nucleus. This subsequently allows for the transcription and translation of the pDNA. Furthermore, due to the morphological changes after activation, we are attempting to generate a cell line which can proliferate, myogenically differentiate, and have efficient transfection with the GET system.

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Gene therapy

PP-438

RNAseq analysis reveals divergent molecular events that direct hBMSCs toward fibrosis or bone regeneration: importance of inflammation regulation and osteoblastic differentiation

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INTRODUCTION: Human bone marrow mesenchymal stromal cells (hBMSCs) associated to biomaterials are currently used in clinic for bone repair. Although results are positive, bone formation kinetics are heterogeneous between patients. In order to understand the cell heterogeneity and to optimize the development of this therapy, it is necessary to better understand the cellular and molecular mechanisms induced by hBMSCs.

METHODS: Cells from 50 donors were grafted in combination with biomaterial subcutaneously in an immunodeficient mouse model and analyzed by histology after 6 weeks, then confirmed using an orthotopic model. To understand why the cells have different bone potential, cells were analyzed for cell survival at 24 hours and from 1 to 6 weeks and for their mechanism of action by RNAseq analysis at 8 hours, 1 and 2 weeks after transplantation. Mice depleted in neutrophils by an anti-Ly6G were also used.

RESULTS: Our results confirm that the bone potentials of the cells is donor-dependent with bone gradient going from no (NB), medium (MB) and high bone (HB) potential. To understand this heterogeneity, we focused on the behavior of hBMSCs and observed that hBMSCs with HB potential were associated with higher cell survival and proliferation after in vivo transplantation. To understand how cell survival is controlled, RNAseq analysis was performed. At 8h, hBMSCs with reduced bone potential strongly express pro-inflammatory cytokines and this is associated with a higher inflammatory response. We then confirm that the long-term survival of hBMSCs depends on their ability to regulate neutrophil activation. Furthermore, RNAseq analysis at 1 and 2 weeks highlights that long-term survival of hBMSCs is associated with increased bone formation only if hBMSCs are able to evolve towards osteoblastic differentiation.

DISCUSSION & CONCLUSIONS: Our results indicate that the HB potential of the cells is due to their ability to survive, proliferate and evolve towards osteoblastic differentiation. This osteoblastic differentiation appears to be a prerequisite for hBMSCs to synthesize the paracrine factors necessary for chemoattraction as well as osteoblastic and osteoclastic differentiation of neighboring cells, otherwise fibrous tissue formation is observed. As our results indicate that the low bone potential of the cells is mainly due to the fact that hBMSCs induce a strong inflammatory response and as we have shown that inhibition of the neutrophilic response improves the survival of hBMSCs as well as bone formation, we propose that anti-inflammatory treatment in parallel with transplantation should improve the clinical protocol.

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Keywords: Cell therapy, Omics / bioinformatics and systems biology

PP-439

Micro-CT Characterisation of a Dual Antimicrobial-Osteoinductive 'Bone Wrap' Applied to a Novel Ex-Vivo Foetal Chick Femur Defect Model

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INTRODUCTION: There are more than 200 musculoskeletal conditions which affect a quarter of the UK adult population. Additionally 300,000 NHS patients acquire a healthcare-associated infection each year¹ most of these are related to an implanted medical device. To reduce the £4.76 billion annual burden these place on the NHS¹, it is necessary to develop materials which aid the repair of tissue and have inherent antimicrobial properties. -NH₂ chemistries have proven to effectively induce osteogenic differentiation of hMSCs² in *in vitro* models. This technology will be used to produce a Bone Wrap – a degradable scaffold designed to work with external fixation to enhance osteointegration whilst minimising bacterial infection. We are optimising a novel *ex vivo* foetal chick femur defect model which utilises μ CT to assess the osteoinductive efficiency of these scaffolds.

METHODS: 3D scaffolds were electrospun from five blends of Gelatine: PCL, then modified to incorporate a Nitric Oxide antimicrobial donor, which reveals an osteogenic amine group when eluted. Femora were removed from chick foetuses after 11 days incubation, a defect was then made in the bone collar region and wrapped with the unmodified scaffold control, while the contralateral bone was wrapped with the corresponding NO-modified blend. Samples were cultured for 14 days before microCT scanning. A Bruker SkyScan 1272 was used for whole bone scans and numerical outputs, and a Zeiss Xradia 620 for visual break site analysis.

RESULTS: The high throughput nature of the SkyScan allows fast whole bone scanning and numerical analysis allowing for large experimental sample sizes, which can be enhanced by the high-quality visualisations possible with the Zeiss Xradia 620 scanner. The data shows the changes in proportion of bony material to cartilaginous material, which can be used to demonstrate the effect that the different scaffold blends, and the Nitric Oxide modification, have on osteogenesis.

DISCUSSION & CONCLUSIONS: Two separate microCT scanning systems have been used in a complimentary way to allow high throughput numerical analysis and high quality representative imaging of the site of interest. μ CT analysis has been proven to clearly show the defect region visually and numerical threshold volume analysis demonstrates the change in osteogenic capabilities exhibited by different material blends and modifications.

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Keywords: Wound healing, In vivo and animal models

PP-440

Evaluation of medium mediated effects of electrical stimulation on osteoblasts

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INTRODUCTION: Physical factors such electrical stimulation can influence cellular behavior and are used in regenerative medicine in many ways [1]. Electrical stimulation can not only be used in heart and muscle stimulation but also in the treatment of bone. Many studies described beneficial effects of electrical stimulation in fracture healing and *in vitro* studies have reported enhanced cell proliferation and collagen production [2, 3]. Treatment with direct contact electrodes can lead to the production of hydrogen peroxide and changes of culture media influence cell fate [4].

METHODS: Human osteoblastic MG-63 cells (ATCC) were cultured in DMEM (10% FCS) without phenol red and pyruvate. Alternating current (AC) electrical stimulation was applied to cells or medium alone for 2 h at 20 Hz, 10 ms rectangular biphasic pulses using L-shaped platinum electrodes [5] under standard culture conditions. Stimulation was current driven at 6 mA. Cells were seeded either in medium before stimulation or in electrically stimulated medium. Cellular reactive oxygen species (ROS) were detected by DCFDA Assay (Abcam). H₂O₂ was quantified by fluorimetric hydrogen peroxide assay kit (Sigma-Aldrich).

RESULTS: The AC electrical stimulation resulted in the generation of about 12 μ M hydrogen peroxide in the culture medium. Subsequently 10 μ M hydrogen peroxide was used as a control for medium-mediated effects. AC-stimulated medium led to an increase in cellular ROS. The electrical stimulation of cells led to an even greater increase but was still overshadowed by the ROS response to 10 μ M hydrogen peroxide.

DISCUSSION & CONCLUSIONS: AC-stimulation influenced the cell culture medium, which in turn increased cellular ROS. The generation of hydrogen peroxide alone is not accountable for the cellular reactions.

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Keywords: Bone and bone disorders (osteoporosis etc), Biomechanics / biophysical stimuli and mechanotransduction

PP-441

Development of a Composite Scaffold for Load-bearing and Biomaterial-controlled Regeneration of Zonal Articular Cartilage Properties

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INTRODUCTION: Articular cartilage degeneration following injury or in diseases such as osteoarthritis (OA), causes significant pain and decreased quality of life. OA affects over 500 million people, with no current treatments able to repair cartilage damage and prevent degeneration¹. Advanced biomaterials have the potential to promote regeneration through provision of instructive cell environments. However, engineering biomaterials recreating the zonal, load-bearing properties of cartilage while providing softer, cell regenerative environments has proven challenging. Additive manufacturing allows engineering of such constructs through precision deposition and integration of biomaterials. Therefore, using fused deposition modelling (FDM) and melt electrowriting (MEW), this study aimed to engineer a cartilage regeneration construct combining softer cartilage-regenerative hydrogels with microfibrous scaffold reinforcement. This approach allows optimisation of regenerative and load-bearing components to support rapid cartilage repair.

METHODS: For load bearing, tri-layered microfibrous scaffolds were fabricated in a range of designs through combined FDM/MEW of polycaprolactone (PCL). The effect of scaffold design and properties on mechanical properties was analysed and best performing scaffolds selected. For regenerative hydrogels, methacrylated gelatin (GelMA) and methacrylated hyaluronic acid (MeHA) blends were produced and chemically characterised (proton-nuclear magnetic resonance). A range of GelMA/MeHA hydrogels were produced and mechanically characterised (rheology, compressive modulus). Best performing hydrogels were selected and their biological performance and cytocompatibility tested in three-dimensional culture with human articular chondrocytes.

RESULTS: Variation of FDM/MEW scaffold design and properties (fibre diameter, deposition pattern) resulted in varied mechanical properties within the range of superficial, intermediate, and deep cartilage zones². Through this process, a continuous layered-PCL scaffold was produced.

GelMA/MeHA blend variation resulted in varying compressive moduli, with highest moduli achieved with highest hydrogel concentrations. Importantly, similar mechanical properties could be achieved with different GelMA/MeHA compositions, permitting fine-tuning of cell-extracellular matrix interactions. Compressive moduli achieved were within optimal ranges for cartilage regeneration. Hydrogel blends all supported high cell viability and differentially affected cell morphology and phenotype in culture.

DISCUSSION & CONCLUSIONS: This work demonstrates the potential for MEW/FDM/Hydrogel composite materials to provide both regenerative cell environments and support load-bearing in cartilage regeneration. Furthermore, this approach allowed tuning of zonal environments within continuous scaffolds across applicable ranges for cartilage regeneration. These variations in

properties and composition were found to impact cell behaviour and phenotypic responses. This construct represents a promising and adaptable approach to regenerate load-bearing cartilage tissue.

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Keywords: Biomaterials, Additive manufacturing

PP-442

Potential synovial fluid biomarkers for progression of knee osteoarthritis using a multiplex ELISA based approach

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INTRODUCTION: In pathogenesis of osteoarthritis (OA), the inflammation is playing a pivotal role. Therefore, understanding the profile of inflammation in a patient population is an essential starting point to predict or prevent OA progression. The synovial fluid (SF) is the most applicable bio-fluid to investigate progression of OA, because of its direct and intimate relationship with different tissues of knee joint. The aim of this study was to identify the profile of selected biomarkers in SF and investigate the correlation according to gender, age and severity of the disease within patients from among the general knee OA population.

METHODS: The concentration of interleukins (IL-6,IL-8), matrix metalloproteinases (MMP-1,MMP-3,MMP-13), MMPs inhibitors (TIMP-1,TIMP-2), COMP and adipokine (adiponectin) was measured and analysed in SF using a multiplex ELISA based approach from 65 OA patients (46 patients with early OA and 19 patients with end-stage OA, according to the Kellgren and Lawrence grading scale).

RESULTS: The concentration level of analysed biomarkers was not significantly different according to gender, except of adiponectin (Median:4753 ng/mL vs. 1325 ng/mL, $P=0.0289$). The levels of MMP-13 and COMP protein in SF were positively correlated with the age of patients ($r=0.2813$, $p=0.0295$ and $r=0.3684$, $p=0.0164$, respectively). We detected no significant difference of IL-6 and IL-8 concentration in early OA SF compared with end-stage OA SF. Nor were any remarkable differences found in concentration levels of these cytokines according to age or gender. Significant differences in the concentration of MMP-3, TIMP-1 and COMP (25054 pg/mL vs. 35705 pg/mL, 12955 pg/mL vs. 15734 pg/mL, 3324 pg/mL vs. 4601 pg/mL, respectively) were detected in the SF of patients with early OA compared to the group of patients with end-stage OA.

DISCUSSION & CONCLUSIONS: To our knowledge, this is the first study in a Slovak population that compares and correlates a profile of synovial inflammatory mediators in SF from OA knee with other important variables such as age, sex and severity of disease. Furthermore, three biomolecules (TIMP-1, MMP-3 and COMP) were identified, that correlate with OA grade and could have the potential of being utilized as a marker, although further research is indicated to resolve the role of the cytokine network in knee OA diagnosis to reach concrete conclusions that can be generalized.

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Keywords: Cartilage / joint and arthritic conditions, Bone and bone disorders (osteoporosis etc)

PP-443

Fabrication of an injectable hydrogel with controllable in situ pore formation for the repair of chondral lesions

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INTRODUCTION: Injectable hydrogels as tissue engineered scaffolds are a promising treatment modality for the repair of cartilage lesions. A key requirement of hydrogels for the repair of chondral lesions is high mechanical strength and stiffness to facilitate biomechanical loading. Stiff hydrogels can recapitulate the mechanical properties of cartilage but coincides with low porosity, limiting the diffusion of nutrients to cells as well as confining the space that cells can proliferate and migrate to [1]. We therefore developed an injectable hydrogel which decoupled stiffness and porosity through addition of hydrolytically degradable alginate microparticles into constructs.

METHODS: Alginate was oxidised by sodium periodate to render it hydrolytically degradable and alginate microparticles were formed via electrospraying. Microparticles were subsequently embedded into a hydrogel composed of gelatin methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA) followed by mechanical and rheological characterisation before and after pore formation. Porogens were stained to assess their degradation kinetics and pore-forming properties. Bovine chondrocytes were stained with DAPI to monitor the proliferation and migration of cells before and after porogen degradation followed by histological analysis to compare cartilage synthesis in constructs with and without pore formation.

RESULTS: Alginate was partially oxidised between 5-10% to generate particles that degrade after 5-7 days. Compression testing of porogen embedded hydrogels showed similar stiffnesses before and after porogen degradation. In vitro cell experiments suggested that incorporation of the MPs into GelMA/HAMA hydrogels promoted the proliferation of chondrocytes. Imaging of stained cells confirmed their migration and spreading to generated pores in the scaffold. An increase in collagen type II and GAG content was found for pore-forming groups in hydrogels ranging from 20 – 70 kPa compared to those without pore forming microparticles.

DISCUSSION & CONCLUSIONS: The size of microparticles facilitated their applicability to be incorporated and injected within hydrogel solutions. Hydrolytic degradation of microparticles led to their dissociation, forming interconnected micropores inside the scaffold. Mechanical compression testing and rheological analysis confirmed the effective decoupling of matrix stiffness and porosity with in vitro studies exemplifying how the addition of porogens to mechanically stiff GelMA/HAMA hydrogels can enhance chondrogenesis and ultimately serve as a promising treatment modality for cartilage lesions.

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Keywords: Hydrogels and injectable systems, Biomaterials

PP-444

Investigating the influence of pore size and matrix stiffness on chondrogenesis within cartilage extracellular matrix-derived scaffolds

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INTRODUCTION: Articular cartilage (AC) regeneration still represents a major challenge in the field of tissue engineering. Extracellular matrix (ECM)-derived scaffolds have been shown to promote tissue-specific stem cell differentiation, pro-regenerative immune responses and the functional repair of a range of different tissue types. However, it remains unclear how the structural and mechanical properties of such scaffolds influences cell fate and ultimate tissue regeneration. The goal of this study was to develop a range of cartilage ECM-derived (cECM) scaffolds with different pore sizes and mechanical properties, and to then assess how such factors regulate the chondrogenic differentiation of bone marrow-derived mesenchymal stem cells (bMSCs).

METHODS: Porcine-cECM was obtained following Browe et al. [1]. Scaffold pore size was altered using different lyophilization processes, while scaffold stiffness was varied by altering the cECM concentration. The mechanical properties of the scaffolds were determined via unconfined step-wise stress-relaxation compression tests and cyclic deformation tests. bMSCs (0.5×10^6 cells/scaffold) were seeded on cECM scaffolds. Cell viability and chondrogenesis were evaluated using a live/dead assay (time points: 1, 7, and 28 days), histology (time points: 1 and 28 days) and biochemical assays to determine sGAG and collagen deposition (time points: 0 and 28 days).

RESULTS: Altering the freezing cycle was found to increase pore size within cECM scaffolds. Furthermore, increasing the ECM concentration within the scaffold was found to increase scaffold stiffness without negatively affecting its elasticity. Cell viability within all cECM scaffold remained high over 28 days of culture. Histological analysis of the bMSC laden scaffolds demonstrated that larger pores supported more homogenous cellular distribution and higher levels of cartilage-specific matrix deposition. Biochemical assays confirmed this finding, with larger pores supporting significantly higher levels of collagen deposition at day 28.

DISCUSSION & CONCLUSIONS: We have developed strategies to vary both cECM scaffold pore size and stiffness, which in turn play a key role in regulating MSC differentiation and cartilage matrix deposition. By modulating scaffold ECM concentration, it is possible to significantly improve the mechanical properties of such implants, thereby addressing a key concern associated with their potential in vivo use. As expected, larger pores supported superior cell migration through the scaffold, higher cell viability and improved chondrogenic differentiation.

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107(10): p. 2222-2234.

Keywords: Cartilage / joint and arthritic conditions, Decellularised matrices

PP-445

Human osteochondral explant tissues as reliable models for ex vivo testing of novel therapeutics in osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a degenerative disease that progressively involves all joint compartments leading to their destruction and loss of function. Currently no disease modifying drug exist for treating OA. Limitations for the development of innovative therapeutics are mainly related to the complexity of the underlying pathogenic mechanisms as well as the unmet need for relevant disease models. Explanted cartilage tissue has been proposed as a modality to study cartilage ontogeny, to understand cartilage repair mechanisms and to test future OA therapies as they accurately reproduce in vivo conditions while avoiding challenges of in vivo experiments

METHODS: Human osteochondral samples- from patients undergoing total knee replacement were kept in incomplete chondrogenic media (ICM) or in serum free DMEM. Explants with or without synovial tissue were established. CM was obtained from human primary ADSC in serum free media. Explant-released cytokines were quantified by. ELISA for Human tumor necrosis factor alpha (TNF α) and interleukin -6 (IL-6) and qPCR-based immunoassay for Human IL-17A and Human IL-1 β (Pro quantum™ immunoassay kit Invitrogen). Histology, Western blot and Immunohistochemistry were performed to detect Collagen type II (Col II) matrix metalloprotease 1 and 13 (MMP I, MMP-13), Perlecan and beta galactosidase (BGAL).

RESULTS: we found that culture media as well as synovial tissue presence influences the level of detectable IL-6, IL-17A; CM increased IL-6 presence up to 29 days; TNF α and IL1 β levels decrease after 7 days in culture and after CM treatment in both DMEM and ICM. Histology revealed presence of active chondrocyte with enlarged hyperchromatic nuclei in CM treated explants while WB and Immunohistochemistry showed MMP 1 and 13 presence merely within untreated explants consistent ColIII and Perlecan within treated explants and absence of BGAL.

DISCUSSION & CONCLUSIONS: Time in culture, type of culture media and synovial presence influence explant reactivity. Presence of synovial tissue increase explant reactivity especially in regard to pro and anti-inflammatory cytokines released by the explanted tissue and should be preferred when an anti-inflammatory effect is expected from the part of proposed therapy. Histology and immunohistochemistry can detect markers of tissue regeneration. Explant culture can serve as a reliable ex vivo models for testing both anti-inflammatory as well as tissue remodeling intervention for treating OA as an intermediary step, potentially reducing the number of animal required for in vivo experiments

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Keywords: Disease models, Cartilage / joint and arthritic conditions

PP-446

Are Cells of intervertebral discs with type 1 Modic Change on MRI showing a history for impaired spinal fusion?

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INTRODUCTION: Type 1 Modic changes (MC1) in the spine on the magnetic resonance imaging are believed to be related to changes in the crosstalk of the intervertebral disc (IVD) with the neighboring bone-marrow of the vertebrae and to contribute to low back pain. Here, we hypothesized that cells of the MC1 discs possess a specific transcriptome that is different to relatively healthy non-degenerative cells coming from trauma.

METHODS: 13 IVDs were sampled with written consent from seven patients, four IVDs from three trauma ("control") and nine IVDs from four chronic low back pain patients diagnosed with MC1. Total RNA was extracted and purified by silica columns from passage 0 cells that were expanded in normoxia in low glucose Dulbecco Modified Eagle Medium (DMEM) and 10% fetal calf serum after mild enzymatic overnight digestion with pronase/collagenase 2. From each IVD, nucleus pulposus cells (NPC) and annulus fibrosus cells (AFC) were sequenced, thus 26 samples. High quality total RNA was prepared for sequencing using the Illumina TruSeq Stranded mRNA Library Prep assay. The samples were processed at the Functional Genomics Center of the University of Zürich and were run on an Illumina sequencer. Routine bioinformatic analyses were conducted.

RESULTS: The differentially expression analysis (DEGs) of the NGS data showed that 811 genes were differently either up or down-regulated comparing the two groups in NPC, 240 in AFC and 83 genes in both, NPC and AFC when comparing four control IVDs compared to the nine MC1 discs. We found among MC1 and control cells that genes were differentially expressed involved in the matrix synthesis (e.g. COL9A3 -4.04 times down in MC1 ($P = 0.00016$)) and in bone metabolism (e.g. SPP1 = secreted phosphoprotein 1 or better known as Osteopontin and SP7, aka Osterix) among many other. We detected DEGs related to matrix production between MC1 and "control" group: COL2A1 $P = 0.0003$ for NPC, and 0.226 for AFC, respectively), SOX9 ($P = 0.004$ for NPC, and $P < 0.0001$ for AFC, respectively). Pathway overrepresentation analysis (PRA) revealed that inflammatory pathways, and pathways in skeletal system development and ossification and cartilage development were significantly down-regulated in MC1.

DISCUSSION & CONCLUSIONS: Pathways of bone turn-over and inflammation were over-represented in the NGS data. We cannot exclude from the current data that MC1 could be a risk factor for failure of spinal fusion.

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Zürich.

Keywords: Bone and bone disorders (osteoporosis etc), Omics / bioinformatics and systems biology

PP-447

Biofabrication of sodium alginate-silicon nitride/poly (vinyl alcohol) composite biomaterials as a shock-absorbing scaffold

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INTRODUCTION: Silicon nitride is clinically used as a bioceramic for spinal fusion cages due to its high strength and osteoconductivity. However, our previous studies have shown that silicon nitride has poor damping properties [1], which may affect the bone formation capacity during vibration or impact. In addition, damping is critical to prevent traumatic bone fracture.

However, there is a paucity of spinal implants that have both good damping performance and an osteogenic effect. Therefore, the aim of this study was to fabricate sodium alginate-silicon nitride/poly(vinyl alcohol) (SA-SiN/PVA) composite scaffolds for use in spinal implants, and more generally in bone tissue engineering applications, and to characterize their damping properties and osteogenic capacity.

METHODS: SA-SiN was produced and was added to a 15 wt.% PVA solution. The mixture was frozen at -20 °C and thawed at room temperature three times to achieve the physical crosslinking of the PVA hydrogel. Subsequently, the samples were then lyophilized and immersed in a 4 wt.% CaCl₂ solution to achieve the chemical crosslinking.

Compression tests were performed on a dynamic testing machine. The cellular response on the scaffolds were evaluated using mouse pre-osteoblasts (MC3T3-E1). Cell adhesion was analysed by Actin/DAPI staining and the expression of osteogenesis-related genes was measured by RT-qPCR.

RESULTS: The elastic modulus of the SA-SiN/PVA scaffold was 2.46 ± 0.24 MPa, which is significantly higher than that of the SiN/PVA scaffold (0.63 ± 0.12 MPa). We also observed that SA-SiN/PVA with double cross-linking exhibited a larger hysteresis loop, indicating a higher energy dissipation capability. In addition, the SA-SiN/PVA scaffold also showed a height recovery rate of more than 95% after being compressed to 67% strain.

Actin/DAPI staining images showed that MC3T3-E1 were attached successfully and proliferated well on the SA-SiN/PVA scaffold. Additionally, the SA-SiN/PVA scaffold stimulated the differentiation of MC3T3-E1 and resulted in higher expression of relevant osteogenic genes compared to SA/PVA scaffolds.

DISCUSSION & CONCLUSIONS: In our study, a novel SA-SiN/PVA composite scaffold was fabricated, which allows for improved energy absorption efficiency and rapid elastic recovery. Additionally, our study showed that the stiffness and recoverable energy dissipation can be significantly improved by combining physical and chemical cross-linking. Regarding the interaction between cells and materials, the results suggest that the addition of silicon nitride stimulated osteoblast differentiation. Overall, the combination of ceramics and tough hydrogels facilitates the development of advanced composites for spinal implants with good damping and osteogenesis.

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REFERENCES: Du et al., Ceramics International, 2021.

Keywords: Intervertebral disc / spine and their disorders, Biomaterials

PP-448

Developing ECM/Chondroitin Sulphate Matrix Hydrogels for Discogenesis

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INTRODUCTION: Degeneration of intervertebral discs (IVD) is believed to be a primary cause of lower back pain with an estimated 80% lifetime prevalence and associated cost of 100 billion dollars each year in the USA alone¹. IVD degeneration progresses in a complex interplay that is not yet fully understood but involves the loss of glycosaminoglycans (GAGs) from the central part of the disc, the nucleus pulposus (NP). GAGs are highly negatively charged polysaccharides bound to a core protein. Specifically, GAGs such as chondroitin sulphate (CS) imbibe water and maintain hydration levels of the NP, while the surrounding annulus fibrosus (AF) tissue restrains the NP's volume. This results in the NP being incompressible and capable of absorbing the compressive load of the spine². GAG loss therefore leads to dehydration and altered biomechanics, resulting in a concomitant shift in NP cell matrix deposition towards the more fibrous type I collagen instead of type II and reduced extracellular matrix (ECM) elasticity³. The objective of this study was to examine the effects of an ECM/CS hydrogel on NP cell viability and matrix production in a 3D *in vitro* setting.

METHODS: NP tissue from bovine coccygeal discs was isolated, cellular components removed and the remaining NP ECM pepsin-solubilised and methacrylated to create an ECM-MA. A methacrylated chondroitin sulphate (CS-MA) formulation was also synthesised. NP cells were encapsulated in different combinations of ECM, ECM/CS or CS only formulations and photo-crosslinked to obtain 3D hydrogels of varying ratio and concentration (w/v). Cell viability and collagen production was assessed over 14 days.

RESULTS: ECM-MA and CS-MA materials were found to remain stable and support cell viability (> 75%) over 14 days. A higher degree of cell death was observed with increasing concentrations of CS up to 20% w/v, which may be due in part to altered osmolarity or charge effects.

DISCUSSION & CONCLUSIONS: NP cells have previously been shown to adopt a rounded morphology and produce increased GAGs and specific collagen types in ECM/CS hydrogels, which simulates the native 3D environment of healthy tissue³. This study provides a new avenue for developing decellularized ECM hydrogels functionalised with GAGs. By elucidating optimal ECM/CS ratios, hydrogels can be developed for applications such as bioprinting tissue analogues for studying regeneration and degeneration processes.

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Keywords: Hydrogels and injectable systems, Biomaterials

PP-449

3D Bio-printed Breast Tumor Spheroid Models for the Evaluation of Chimeric Antigen Receptor (CAR-T) Cell Therapy

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INTRODUCTION: Immunotherapy is a promising treatment to increase the survival and quality of life of patients with triple negative breast cancer¹. Novel immuno-oncology approaches are traditionally evaluated in two-dimensional (2D) culture models, which has been shown to disregard cell-cell and cell-extracellular matrix interactions, thus, failing to reproduce in vivo interactions accurately². In this regard, 3D spheroid models have been reported to display superior immune-modulation, activation and proliferation of cancer cells, resulting in a better representation of the tumor microenvironment (TME)³. Although a wide range of bio-inks have been reported for the growth of breast cancer spheroids, the reports of T cell bio-printing are limited to their expansion rather than their use in co-culture models⁴. Reported 3D co-culture systems are either co-printed or use spheroids in suspension⁵, thus, T cell infiltrative and motility properties, which are crucial for clinical therapy efficacy, are disregarded. Therefore, this project evaluates existing chimeric antigen T cell therapy using a 3D bio-printed breast cancer tumor spheroid model; whilst, delving deeper into understanding how bio-ink formulation influences the infiltration and motility of human T cells.

METHODS: For the proposed model, spheroids were pre-formed and later embedded within several gelatin alginate bio-ink formulations (5% gelatin:1-2% alginate, 7%gelatin:1-2% alginate) resembling the components of the ECM. Cell viability, spheroid size was monitored for a 14-day period using image-based approaches. Human T-cells were bio-printed in the same bio-ink formulations and tested for cell viability, infiltration and motility through image and flow cytometry-based approaches.

RESULTS: Viability of both cell types was lower when increasing the ratio of alginate within the bio-ink. Similarly, motility and infiltration of T cells were improved in bio-inks with lower gelatin and alginate ratios. Pre-formed spheroids were shown to be compact, spherical and of a consistent spheroid size, 750-850 µm in diameter, and retained their shape and size after 8 days of transfer.

DISCUSSION & CONCLUSIONS: In conclusion, softer bio-inks with a lower concentration of gelatin and alginate, such as 5% gelatin:1% alginate, displayed higher cell viability of both cell types, improved spheroid growth, human T cell motility and infiltration within a 3D environment; Thus, deemed to be suitable for a 3D co-culture.

ACKNOWLEDGEMENTS: Perriman Group, the Armstrong Group and the Wolfson Bioimaging Facility.

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Keywords: In vitro microenvironments, Cell therapy

PP-450

Modelling the Extracellular Matrix Complexity in a Multi-Compartmental Biomaterial based Multicellular 3D Pancreatic Cancer Model

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INTRODUCTION: With a 5-year survival rate of only 11%, Pancreatic Ductal Adenocarcinoma (PDAC) is the 7th leading cause of cancer related deaths worldwide. This is partly attributed to the tumour microenvironment (TME) and its resistance to treatment. The TME consists of various structural, cellular and protein components. Advanced study of the disease and its treatment resistance mechanisms, require the development of biomimetic, *in vitro* tumour models. We have previously developed a poly urethane (PU) based 3D pancreatic cancer model using (i) pancreatic cancer cells (monocellular model) and (ii) pancreatic cancer cells, stellate cells and endothelial cells, all of which are important elements of the pancreatic cancer cellular TME (multicellular model). We have shown long term physiological maintenance, feasibility of extracellular matrix (ECM) mimicry, mimicry of PDAC fibrosis/desmoplasia, and we have mapped effect of hypoxia(1-4).

The current work focusses on further development of our scaffold assisted multicellular model via optimisation of the ECM composition for the different cell compartments of our model (cancer vs stromal).

METHODS: PU scaffolds were prepared as per previously published protocols(1-4). Absorption based surface modification of the scaffolds enabled coating with ECM proteins (laminin, collagen I and/or fibronectin) followed by incorporation of different cell types (cancer, stellate, endothelial) within the equivalent stromal (external) and cancer (internal) compartments. The effect of ECM coatings and their combinations for both model compartments were systematically assessed (> 4 weeks). Imaging of cellular proliferation/spatial organisation and ECM secretion was carried out along with q-PCR assessment of various biomarkers, e.g. EMT or metastatic markers.

RESULTS: Our study shows that cancer cell and stromal cells show preference to different ECM proteins (including ECM combinations) for optimal growth. Furthermore, different ECM combinations can affect the cell spatial distribution, ECM secretion and biomarker upregulation.

DISCUSSION & CONCLUSIONS: Our data show the importance of ECM proteins tailoring and combination for the growth, proliferation and cellular protein and biomarker secretion for cancer and stromal cells *in vitro*. Additionally, our work highlights the importance of developing zonal/spatial cellular and matrix structures in 3D for the accurate mimicry of the tumour tissue architecture.

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Keywords: Disease models, In vitro microenvironments

PP-451

Investigating the Impact of Hypoxia on Radiotherapy Response of a 3D Scaffold Based Pancreatic Cancer Model

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INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is a cancer of unmet clinical needs. Non-specific symptoms, high metastatic occurrence and treatment resistance results in extraordinarily low survival rates. PDAC encompasses a unique tumour microenvironment with hallmarks including desmoplasia and tumour hypoxia, known to impair treatment responses. Radiotherapy for PDAC is traditionally controversial with contradictory clinical trial outcomes highlighting a need for further understanding and optimisation of this treatment option. We have previously developed a polymeric scaffold assisted PDAC model that could be cultured long term (>4 weeks), showed high cell proliferation, ECM protein secretion, hypoxic gradients mimicry, The model was used for chemoradiotherapy assessment (at normoxia (1-2).

In this work, utilising our PDAC model, we aim to investigate hypoxia-induced radio-protection for pancreatic cancer and its effect on cell proliferation and ECM protein secretion.

METHODS: Polymeric scaffolds were prepared as per previously published protocols (1-4).

Absorption based surface modification of the scaffolds enabled coating with fibronectin, for ECM mimicry. Pancreatic cancer cells were cultured in 3D under hypoxia (5% oxygen) followed by radiation exposure (6 Gy) and were compared to a normoxic control (21% oxygen) (3). Post treatment analysis was carried out at various time points to assess cellular organization, viability and mapping of radio-protection via immunofluorescence staining of live/dead, Caspase 3/7, HIF1- α , actin and collagen I.

RESULTS: This research provides a platform for radiation responses in a 3D hypoxic PDAC system. Generally, low oxygen levels lead to high treatment resistance, i.e., higher viability and lower apoptosis post-radiotherapy. Moreover less post-radiotherapy disruption of the formed ECM (collagen) was observed in 3D models cultured in hypoxia.

DISCUSSION & CONCLUSIONS: Our data show for the first time (i) a 3D polymeric scaffold supporting long-term hypoxic PDAC cell culture (ii) a long-term post-treatment in situ cell characterisation for radiotherapy treatment patterns. Furthermore, we mapped hypoxic induced radioprotection in the scaffold, indicating a more realistic biomimetic research platform. Overall, this system provides a versatile platform to study hypoxia-associated radio-resistance profiling of PDAC

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Keywords: Disease models,

PP-452

2D and 3D in vitro study of carbohydrates-modified Pt(IV) pro-drugs: a novel therapeutic strategy to target Osteosarcoma

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INTRODUCTION: The selectivity vs. cancer cells has always been a major challenge for cisplatin-based anticancer drugs for the treatment of several tumors¹. One strategy to overtake this challenge is to modify the coordination sphere of the metallic centre with specific vectors whose receptors are overexpressed in cancer cell membrane, such as sugars, in order to create a targeted metal-based anticancer drug. Moreover, Pt(IV) functionalized with sugars show several advantages with respect to Pt(II) counterparts, being more stable and less prone to substitution reaction². Here, we report the synthesis of various mono glyco-modified Pt(IV) pro-drugs based on cisplatin scaffold and functionalized with carbohydrates (e.g., glucose and galactose), named complexes, and their biological evaluation in vitro on bi-dimensional (2D) cell cultures of MG63, SAOS-2 and U2-OS osteosarcoma cell lines as well as on enriched-CSCs (Cancer Stem Cells), compared to standard cisplatin. The biological effect of the complexes was evaluated also on in vitro three-dimensional (3D) scaffold-based models of osteosarcoma as more predictive tumour microenvironment.

METHODS: The complexes were tested at different concentrations under in vitro 2D conditions in order to select the best ones for the evaluation on in vitro 3D scaffold-based model of osteosarcoma. Enriched-CSCs were obtained by sarcosphere-forming culture. Cell response was investigated in terms of cell viability (MTT assay), cell morphology (Actin and DAPI staining) and cellular internalization of Pt ions (Inductively Couple Plasma – Optical Emission Spectrometry) on the complexes on in vitro 2D and 3D conditions.

RESULTS: Compared to cisplatin, the complexes showed a significantly higher cytotoxicity, particularly on MG63 cell line. A statistically significant higher cytotoxicity was observed also on enriched-CSCs, strictly related to the increased platinum uptake by cells, compared to cisplatin. The morphological evaluation confirmed the cytotoxicity results and showed a reduction of the dimension of the spheres confirming the cytotoxicity results.

DISCUSSION & CONCLUSIONS: The presence of a monosaccharide increased the anticancer effect and the drug internalization on osteosarcoma cell lines and enriched- CSCs. The discrimination between glucose and galactose is not evident confirming the hypothesis that the complexes are internalized by passive diffusion. The in vitro results on deprotected complexes and 3D scaffold-base models is work in progress.

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Keywords: Cancer, In vitro microenvironments

PP-453

Targeting FSP1 to sensitize human osteosarcoma to ferroptotic cell death

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INTRODUCTION: Ferroptosis is one of the newly discovered forms of regulated cell death, which differs from apoptosis, necrosis or autophagy, at both morphological and biochemical levels. The molecular mechanism of ferroptosis execution relies on the iron-dependent intracellular generation and accumulation of phospholipid hydroperoxides (PLOOHs or lipid-ROS), the main executioners of this form of cell death [1]. However, cancer cells evolved several strategies to sustain survival and cancer growth, with the FSP1-dependent CoQ10 reduction system with antioxidant properties representing a recently described anti-ferroptotic pathway [2]. Interestingly, increasing data indicate an inverse sensitivity of many tumors to apoptosis and ferroptosis, with apoptosis-resistant tumors being sensitive to ferroptosis and vice versa. Therefore, although the sensitivity to ferroptosis is highly heterogeneous in different cancer types, this new form of cell death offers new therapeutic opportunities [1].

METHODS: Susceptibility to ferroptosis was evaluated in a panel of osteosarcoma (OS) cell lines: U2OS, MG-63, and HOS. Cells were exposed to the ferroptosis inducer RSL3 alone or in combination with an FSP1 inhibitor (RSL3+iFSP1). Cell viability was evaluated by staining cells with AlamarBlue® after 18h of treatment. The basal levels of FSP1 expression in OS cells was evaluated at both mRNA (by qRT-PCR) and protein levels (WB analysis). The Calcein-AM/PI staining was used to evaluate sensitivity of cells cultured in 3D models upon RSL3 and iFSP1 exposure.

RESULTS: Our results revealed a heterogeneous sensitivity of OS cells to ferroptosis execution, with U2OS representing the most resistant while HOS as the most sensitive. Interestingly, basal levels of FSP1 expression correlated with OS susceptibility to ferroptosis. Indeed, inhibiting the activity of this factor, by the specific inhibitor iFSP1, we re-sensitized all OS cells to ferroptosis. Moreover, these results were confirmed in 3D OS cell cultures as well.

DISCUSSION & CONCLUSIONS: OS cells are characterized by heterogeneous sensitivity to ferroptosis with basal expression of FSP1 representing a good predictive biomarker. Importantly, FSP1 might represent a new valuable therapeutic target since OS sensitivity to pro-ferroptotic drugs can be efficiently restored by using FSP1 inhibitors.

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Keywords: Bone and bone disorders (osteoporosis etc), Cancer

PP-454

Human breast cancer on-chip for personalized therapy

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INTRODUCTION: Breast cancer is one of the most common tumors worldwide featured by heterogeneity. The expression of hormone receptors marks different breast cancer subtypes. Each type is pharmacologically treated by using molecules targeting hormone receptors, but this frequently drives to chemoresistance. For this reason, the possibility to replicate this hostile human pathology in vitro represents an urgent need to understand the biological basis behind heterogeneity and chemoresistance, and explore the efficiency of pharmacological treatments.

METHODS: We developed a breast cancer micro-tissue model (BC-μTP) by using an established bioengineering strategy (1) starting from primary cells extracted by human biopsy. Specifically, cancer associated fibroblasts were seeded on porous gelatin micro-scaffolds and cultured in dynamic condition in spinner flask. Fibroblasts produced their own extracellular matrix (ECM). Afterwards, breast tumor cells were seeded on the formed micro-tissues and moved into a microfluidic device for dynamic culture, live monitoring and drug administration.

RESULTS: Breast cancer associated fibroblasts adhered on the scaffolds and produced their collagenous ECM, already after 5 days of culture. Matrix secretion increased from day 5 to day 21 in spinner flask, and went hand in hand with scaffold degradation. Tumor cells adhered on micro-tissues and developed clusters with heterogenous morphology. Breast cancer microtissues were cultured on-chip for 3 weeks. We observed the dynamic culture enhanced tissue viability and preserved the ECM from degradation over long time culture.

DISCUSSION & CONCLUSIONS: The BC-μTP on-chip aims at replicating the main features of the primary tumor. We already observed model histocompatibility and we are going to further investigate tissue morphology and transcriptomics. The microfluidic chip allows the dynamic culture of the tissue. Moreover, it will guarantee a spatiotemporal control over drug release and tissue monitoring. We argue that BC-μTP treatment on-chip may respond to clinical outcomes (in terms on number of proliferating cells and mass volume). In this way it may be considered as a predictive model, suitable to optimize patient specific therapy.

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REFERENCES: C. Mazio et al. Acta Biomaterialia, 2018

Keywords: Cancer, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

PP-455

Effect of Age-specific Decellularized Extracellular Matrix on Neuronal Physiology and Repair

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INTRODUCTION: Spinal cord injury poses major limitations on an individual's function and control of movement. The extracellular matrix (ECM) is a critical and often overlooked component of the resident microenvironment of the central nervous system (CNS) that plays a pivotal role in neuronal development, growth, maturation, signaling, ageing and injury (1). Developing reliable therapeutic methods has been a major challenge due to the complex and dynamic ECM microenvironment after injury. In this study we propose to interrogate the ability of fetal, postnatal, adult decellularized ECM (dECM) to facilitate the growth and functional maturation of induced pluripotent stem cell (iPSC) derived motor neurons (MNs) grown in vitro and in vivo.

METHODS: We created natural ECM matrices from isolated mouse spinal cord tissue from neonatal (postnatal day 0) and adult (16-20 weeks old) mice. The decellularized tissue was subsequently freeze-dried, digested, and re-solubilized, yielding a natural, dECM solution that we used as a natural spinal cord matrix to culture iPSC-derived MNs. To characterize the effect of age-specific dECM in response to SCI, we will utilize a robust traumatic injury model where severe contusive injury will be performed at the T10-T11 spinal level of mice.

RESULTS: We found that MNs plated on the adult-derived spinal cord dECM in vitro exhibited a range of morphological, synaptic, and electrophysiological elements of advanced maturation compared to MNs plated on neonatal dECM or on a purified laminin coating. MNs grew larger in size, developed a more elaborate network of processes, upregulated pre- and post-synaptic proteins, more MNs fired spontaneous action potentials (AP), exhibited more mature AP slopes with a shorter half-width, and a faster rate of rise and fall.

DISCUSSION & CONCLUSIONS: Collectively, these experiments demonstrate the pronounced effects of dECMs cues on neuronal development and physiology and rendered credibility to the hypothesis that ECM signals can differences in functional neuronal maturation. This project will go beyond of the state of the art and will elucidate new mechanisms in the engineered materials as well as in the physiopathology of the MNs that will make this therapy a promising tool and a focus of research for SCI.

ACKNOWLEDGEMENTS: We are grateful to the following funding sources: Mike Lane fellowship from Castellars de Vila de Gracia (CVG) 2021 (Z.A) and Grant PID2021-124839OA-I00 funded by MCIN/AEI/10.13039/501100011033.Ramon y Cajal fellowship RYC2020-028732-I (Z.A), Erasmus+ TRAINEESHIP 2022-2023 (N.L).

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Keywords: Nervous system (brain-central-peripheral / disorders), Decellularised matrices

PP-456

Optimization of the Nichoid micro scaffold for the expansion culture of human adipose-derived mesenchymal stem cells

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INTRODUCTION: Adipose-derived mesenchymal stem cells (hADMSCs) are a promising resource for regenerative medicine, and they can be harvested directly from the patient with moderately invasive procedures and re-implanted without eliciting immune response [1]. Several clinical trials are active in phase II-III, but new technologies are requested to develop more efficient therapies. In this context, the micro-scaffold "Nichoid" has shown to induce pluripotency in stem cells, thanks to its peculiar 3D structure [2,3]. The aim of this study is to design, fabricate and validate a new geometry of the Nichoid, optimized for the expansion of hADMSCs and to investigate the mechanotransduction process of cells in different scaffold configurations, using a combined computational and experimental approach.

METHODS: We developed a computational model of the scaffold structural deformations caused by cell adhesion, using COMSOL Multiphysics and we designed a new "cubic" scaffold pore architecture that optimizes the mechanical stimuli exerted by the scaffold deformation on the adhering cells. Cubic Nichoids were fabricated by two-photon polymerization technique using a biocompatible photoresist as already described [4]. To validate the cubic Nichoid, we performed several biomolecular and bioinformatics analyses on hADMSCs expanded inside the scaffolds: a metabolic assay, RNA-sequencing, Real Time PCR, Western Blot, and immunofluorescence.

RESULTS: We predicted computationally the structural stability and the maximum deformation for several pore dimensions (10x10um, 20x20um and 30x30um). We identified the most suitable structure for hADMSCs as the one that maximized the isotropy of forces acting on cells. We observed a more roundish nuclei in cells cultured on the cubic Nichoid, with respect to traditional Nichoids. This corresponded to a higher expression of pluripotency genes induced by mechanical stimuli, also verified by the up-regulation of the stemness transcriptional core.

DISCUSSION & CONCLUSIONS: The cubic Nichoid recreated an ex-vivo physiological environment optimized for hADMSCs. This new scaffold demonstrates how engineering technologies can support regenerative medicine and in particular the clinical translation of stem cell therapies. Indeed, cells expanded in this new scaffold not only showed an increased maintenance of function, they could also be easily collected after expansion culture in view of an autologous transplantation and thus represent a valid therapeutic stem cell approach.

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Keywords: Microenvironment and niche engineering,

PP-457

NGF in combination with Hyperbaric oxygen treatment promotes neuronal survival and axonal regeneration

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INTRODUCTION: Hyperbaric oxygen (HBO) is a promising therapeutic approach to promote peripheral nerve regeneration. Underlying mechanisms of action are poorly understood resulting in inconsistent treatment protocols and unclear indications. Within this context, the present study evaluated the effects of single or repeated HBO treatment sessions alone or in combination with NGF on PC12 cell. Cell proliferation, survival and differentiation (neurite outgrowth) were analyzed as an outcome measures.

METHODS: PC12 cells were exposed to single or daily session of HBO (90min) over four days. Five groups were studied with or without NGF (nerve growth factor) stimulation: control (21% oxygen), 100% oxygen at 1.0 ATA (atmosphere absolute), 2.0 ATA, 2.5 ATA or 3.0 ATA. Brightfield imaging, live/dead assay, cell proliferation using alamar blue reagent and axonal outgrowth using immunostaining of tubulin β -III were performed.

RESULTS: Single dose of HBO treatment enhanced the proliferation of unstimulated PC12 cells at all pressure conditions in contrast to repeated HBO treatment ($p < 0.01$). Apposing to the cytotoxic effects of repeated HBO treatment, NGF-stimulated PC12 cells showed significant neuroprotection and axonal outgrowth as evidenced by tubulin β -III staining. However, 3.0 ATA impeded axonal outgrowth in all the groups.

DISCUSSION & CONCLUSIONS: NGF exhibited neuroprotective effects against oxidative stress mediated cell death in PC12 cells. Further, combination of NGF and HBO treatment (1-2.5 ATA) resulted in significant axonal outgrowth. These results hold a great promise for neural repair and regeneration applications. Further research is needed for investigating the underlying mechanisms of NGF-HBO mediated functional effects in vitro and in a rat model of sciatic nerve injury.

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Keywords: Nervous system (brain-central-peripheral / disorders), Trauma / surgery and rehabilitation

PP-458

Using adipose stem cell spheroids with fibrin hydrogel-assisted delivery for nerve repair: an experimental study

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INTRODUCTION: Current treatment options of peripheral nerve injuries, despite many surgical improvements, are still unsatisfactory. One promising method is the combination of fibrin-hydrogel nerve conduits (FNCs) with human adipose stem cells (hASCs). The FNCs, as a nerve guidance channel combined with hASCs, as supportive cells releasing neurotrophic factors (NTFs), have shown promising results in vitro and in vivo. Several reports showed that stem cells cultured in spheroids have improved their cytokine profile. However, the therapeutic effect of hASC based spheroids on nerve regeneration is unclear. Thus, the purpose of this study is to establish hASCs spheroids, and evaluate them in vitro and subsequently in animals.

METHODS: Spheroids based on hASC (passage 4 to 8) were generated using agarose microwell mould. To determine the ideal spheroid size and growth kinetics and cell viability, various seeding densities (i.e., 1000 or 2000, 3000 and 4000 cells/spheroid) were assessed for seven days measuring the size and intracellular ATP levels.

RESULTS: hASC spheroids have been successfully established. As expected, the size and the ATP levels were well correlated to the cell density. After a 24-hour period of compaction, the size of the spheroids and the ATP level remained stable over 7 days for low-density spheroids. For the highest density (4000 cells per spheroid), the size and the ATP levels showed a marked decline in the last days of culture (day 6 and 7).

DISCUSSION & CONCLUSIONS: We successfully established optimal growth conditions for hASC spheroids for seven days. Monitoring the growth of spheroids with their size and their ATP levels allows to detect early changes in spheroid viability. To further validate this model, NTFs expression profile will be analysed. Subsequently, we plan to use hASC spheroids combined with FNCs as delivery method in an axonal growth assay in vitro and in a rat model of sciatic nerve gap injury.

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Keywords: Cell therapy, Microenvironment and niche engineering

PP-459

Modeling ischemic stroke in a triculture neurovascular unit on-a-chip

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INTRODUCTION: In ischemic stroke, the function of the cerebral vasculature is impaired. This vascular structure is formed by the so-called neurovascular unit (NVU). The NVU is crucial in maintaining brain homeostasis and healthy functioning of the central nervous system (CNS). A better understanding of the mechanisms involved in NVU dysfunction and recovery may lead to new insights for the development of highly sought therapeutic approaches. To date, there remains an unmet need for complex human in vitro models of the NVU to study ischemic events seen in the human brain.

METHODS: We here describe a human NVU on-a-chip model using a platform that allows culture of 40 chips in parallel. The model comprises a perfused vessel of primary human brain endothelial cells in co-culture with induced pluripotent stem cell derived astrocytes and neurons. The model displays expression of endothelial adherens- and tight junction proteins as well as astrocytic and neuronal markers. In addition, the model presents with relevant brain endothelial transporters and shows spontaneous neuronal firing. Tight barrier function was evidenced by retention of the small molecule sodium fluorescein in its lumen and allows study of compound-induced barrier disruption.

RESULTS: Ischemic stroke was mimicked in the NVU model using a three-fold approach that combines chemical hypoxia, hypoglycemia, and halted perfusion. The resulting cultures showed reduced BBB integrity, lowered mitochondrial membrane potential, and reduced adenosine triphosphate levels, which are common features of ischemic stroke.

DISCUSSION & CONCLUSIONS: The NVU on-a-chip model presented here can be used for fundamental studies of NVU function in stroke and other neurological diseases and for investigation of potential restorative therapies to fight neurological disorders. Due to the platform's relatively high throughput and compatibility with automation, the model holds potential for drug compound screening.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Nervous system (brain-central-peripheral / disorders)

PP-460

Enzymatically crosslinked Silk and Hyaluronic acid based hydrogels for engineering artificial corneal tissue

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INTRODUCTION: The cornea is a multi-cellular component of the ocular surface and is composed of three main cellular layers: epithelium, stroma and endothelial layer. Corneal disease affects millions of people worldwide and in many cases requires the transplant of cadaveric corneas (keratoplasty). Keratoplasty usually has a high rate of success, with almost 93% well-functioning after one year and 74% after 5 years [1]. However, the limited number of qualified donor corneas for transplantation and possible graft rejection makes it necessary to develop alternative treatments. Keratoprosthesis replaces cornea with transparent polymers but are associated with complexity like an increased risk of glaucoma and corneal melt [2]. Tissue engineering approaches allows prompt availability of corneas by using transparent biomaterials with patients' cells, recreating in vitro corneas identical to the native ones. This would not only increase the availability of corneas for keratoplasty but would solve the risk of immune reactions towards the graft.

Hyaluronic acid (HA) is an FDA-approved biopolymer for medical uses, already used for the treatment of eye diseases. Preliminary results from our group on cell adhesion and degradation test of tyramine modified HA (HAT) hydrogels suggest the use of HAT as the base material for cornea tissue engineering. Silk hydrogels have been used for in vivo implantation of partial corneal tissue in rabbits, showing good biocompatibility and biodegradability [2]. Here, we test feasibility of enzymatically crosslinked silk and HAT polymers to form biocompatible hydrogels for growth of corneal epithelial cells (HCLE) and fibroblast cells to form a two-layer artificial corneal tissue for implantation.

METHODS: Different concentration of silk and HAT were crosslinked with Horse radish peroxidase (HRP). Gelation kinetics and swelling behaviour were characterised for 1-week. Preliminary cytocompatibility tests (Alamar blue proliferation, LDH cytotoxicity) using HCLE cells was tested with for 1-week.

RESULTS: Based on the mechanical properties, the gelation time and swelling behaviour, four hydrogel compositions were selected. Biocompatibility tests suggest that the hydrogels support HCLE cell growth.

DISCUSSION & CONCLUSIONS: Further characterization of biomaterial degradation and cell marker expression and inclusion of the second layer would report on the usage of this new biomaterials as artificial corneal tissues.

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Keywords: Biomaterials, In vitro microenvironments

PP-461

Development of Artificial Retinas with Conjugated Polymer Nanoparticles (CPNs) for the Treatment of Degenerative Eye Disease and the Augmentation of Human Vision

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INTRODUCTION: Nowadays, the increasing global prevalence of different types of retinal diseases such as retinal pigmentosis and age-related macular degeneration (AMD) is an essential challenge for regenerative medicine. Moreover, the present trend of the ageing population requires appropriate effective systems of retina implants to restore vision. Current approaches are based on the photoactive inorganic semiconductors, but they have several limitations such as requirements of the complex electrical circuit, additional external power supply and usually non-biocompatible rigid inelastic structure.

METHODS: Consequently, the main aim of the planned research is to develop a novel approach to functional eye retinal prostheses based on the application of organic semiconductors using photosensitive conjugated polymer nanoparticles (CPNs) with the light absorption relating to the native photoreceptors rods and cones. The CPNs have desirable optoelectronic properties such as high extinction coefficient, low photobleaching, and resistance for photodegradation.

The process of the synthesis of the CPNs through the Suzuki-Miyaura cross-coupling polymerisation via miniemulsion for four types of the selected materials corresponding following receptors PFDTBTA for rods, PFO for blue S-cones, P3HT for green M-cones, and p(BODIPYalt-FL) for red L-cones.

RESULTS: According to the received results, it was conducted optimization process of the polymerisation to obtain CPNs with average size below 100 nm, and more than 15 kDa of an average molecular weight. Furthermore, the biocompatible aqueous inks with incorporated CPNs are deposited with selected patterns by the ink-jet printing onto the flexible polymer substrate to deliver final soft organic electronic devices. The performance of these devices to detect incident visible light with high photosensitivity could give an ability to transduce the optical input into an electrical signal to trigger retinal neurons, hence deliver an optical nerve response. The final printed on array platform mimic the structure of the natural human retina and enable visions of different colours.

DISCUSSION & CONCLUSIONS: The proposed system can significantly improve the biocompatibility with retina ganglion cells (RGCs) with relatively low stiffness in comparison to the current epi- and sub-retinal silicon photodiode retina prosthesis. In addition, the performed study could be potentially used in the future soft organic retina implant with NIR or UV absorbing chromophores, which allows obtaining the continuous hyperspectral imaging response of the detected light with different wavelengths of the visible region, and it can considerably enhance the neural stimulation to improve visual perception.

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Keywords: Polymers - natural / synthetic / responsive, Eye

PP-462

A combination of Melt Electrowriting and solution electrospinning to mimic the structure of the human trabecular meshwork

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INTRODUCTION: The human trabecular meshwork (HTM) is a three-layered porous tissue[1] which regulates intraocular pressure (IOP). Changes in HTM thickness, porosity and rigidity[2] hinder the permeability of the tissue increasing IOP, main risk factor for glaucoma development[3]. The physiological mechanisms that cause its malfunctioning remains unclear, and the lack of appropriate in vitro HTM models makes the research even more challenging. Herein, a combination between solution electrospinning (SE) and melt electrowriting (MEW) is explored as a biofabrication technique to produce fibrillar, porous scaffolds that mimic the multi-layered, gradient structure of the native HTM.

METHODS: PCL 10 % nanofibers were fabricated via SE. These nanofibers imitate the innermost layer of the HTM (the most compacted one). Using PCL, different architectures were printed with MEW on these SE nanofibers to recreate the middle and outermost layers. Two architectures were created (circular and triangular) to replicate the central and external layers. Each layer, as well as the SE and MEW combination, were analysed under SEM and mechanically tested. Finally, human trabecular meshwork cells (HTMC) were cultured to evaluate scaffold suitability.

RESULTS: SE scaffolds showed fibre diameters of 770 ± 0.172 nm, pore areas of 5.59 ± 0.28 μm^2 , and elastic moduli of 0.15 ± 0.05 MPa. All MEW scaffolds presented fibre diameter around 30 μm and thickness' between 28 and 88 μm . Pore sizes vary from 546.407 ± 62.03 μm to 1.97 ± 0.92 mm and the scaffolds tensile modulus between 0.014 ± 0.002 MPa and 0.0596 ± 0.02 MPa were obtained. These values depend on their geometry and the number of stacked layers. Primary HTMC attach to the scaffolds, proliferate, and form a confluent layer within 14 days.

DISCUSSION & CONCLUSIONS: The present work demonstrates the utility of SE and MEW combination for reconstructing complex morphological features such as the HTM. Scaffolds with graded porosity showed relevant mechanical properties and supported HTMC culture, observing cell attachment, proliferation, and morphologies close to that in the native HTM. This study provides a biomimetic functional HTM engineered scaffold to improve understanding of structure-function relations in layered tissues.

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Keywords: Additive manufacturing, Biomaterials

PP-463

***In vitro* characterization of different human tissue-engineered skin substitutes composed of human plasma as main biomaterial and their manufacturing process: in search of personalized therapies for dermatological diseases**

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INTRODUCTION: The use of human tissue-engineered skin substitutes (hTESSs) as advanced therapy is a promising strategy in the field of dermatology. Many different models have been or are still under research, evaluating aspects such as cell composition, dermal matrix structure or culture methodologies. The use of human plasma as main biomaterial, comprised of different biomolecules and growth factors, plays a crucial role in cell metabolic activities and wound healing's potential. In this research, we studied several biomaterials combined with human plasma as dermal matrix; compared the use of different cells to produce hTESSs and evaluated the effect of the dehydration process, required to achieve the proper skin substitute's structure, on cell viability.

METHODS: 7 dermal matrices, based on the use of human plasma (Fibrin) as main component, were fabricated: 1)Fibrin-Serine, 2)Fibrin-Fibronectin, 3)Fibrin-Collagen, 4)Fibrin-Laminin (A), 5)Fibrin-Laminin (B), 6)Fibrin-Hyaluronic Acid and 7)Fibrin only. For each, 4 different types of hTESSs were fabricated: Trilayer (adipose tissue mesenchymal stem cells, fibroblasts and keratinocytes), Bilayer (fibroblasts and keratinocytes), Monolayer (keratinocytes) and Control (without cells). Cell metabolic activity (PrestoBlue™) and secretion of different growth factors and cytokines (bFGF, VEGF-A, EGF and CCL5) were measured at the end of the manufacturing process. Cell viability (LIVE/DEAD®) was analyzed before and after dehydration process and immunohistochemistry was performed. Cells were isolated from 7 different human skin samples.

RESULTS: All biomaterials were biocompatible; however no significant differences were observed between them in any of the assays performed. Regarding to cell composition, cell metabolic activity and viability was significantly higher in Trilayer and Bilayer hTESSs ($p < 0.0001$). In the same way, secretion of VEGF and bFGF was increased in Trilayer and Bilayer hTESSs ($p < 0.0001$) but CCL5, a pro-inflammatory marker, was significantly reduced compared to Monolayer and Control hTESSs ($p < 0.05$). Finally, dehydration process significantly reduced viability ($p < 0.0001$).

DISCUSSION & CONCLUSIONS: The presence of human plasma as the main component is responsible of the *in vitro* properties of the hTESS model. It would be interesting to be able to pre-characterize the human plasma used for the manufacturing process, to establish minimum standards that help to increase the clinical success of hTESSs. Regarding to cell composition, those substitutes that resemble native human skin properties in a better way are those composed of three layers. Dehydration process reduces cell viability; but clinical handling is improved. Future research should focus on *in vivo* studies, where the role of the different biomaterials and cells is more determining to promote wound healing.

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Keywords: Hydrogels and injectable systems, Wound healing

PP-464

Advanced wound dressing with gelatin methacrylate (GelMA) hydrogel for skin tissue engineering

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INTRODUCTION: Effective wound healing requires quick wound closure, less scarring, and accelerated angiogenesis. Hydrogels stand out among dressing materials and offer a number of benefits that promote wound healing because of their high biocompatibility, structural resemblance to the extracellular matrix (ECM), and highwater content. Despite the fact that hydrogel is frequently employed in tissue engineering, it is not the best option because of its weak mechanical and vascularization capabilities. Here, we coated GelMA hydrogel on silicone polymer. GelMA hydrogel mixed with amniotic membrane extract increased angiogenesis properties of the wound area.

METHODS: A 1:1 mixture of pharmaceutical-grade two-component A&B silicone was prepared. It was then put into polymethyl methacrylate (PMMA) molds, heated for 5 minutes at 120 °C to form a silicone film. Pharmaceutical grade GelMA (10% concentration) was prepared, then 0.1 mg/ml amniotic membrane extract, Irgacure 2959 were added, the resulting solution was poured onto the silicone film and exposed to UV (at 365 nm) light for 1 minute.

Structural tests including SEM and tensile test were carried out to prove the strength of the scaffold used. The amount of tube formation was examined in order to study the angiogenesis.

RESULTS: The results of this study showed that the silicone structure has no pores and its surface is completely smooth and has no cracks in the structure. The GelMA 10% structure has suitable pores with a size of 65 micrometers. Biodegradability tests showed that the thin layer of GelMA starts to degrade and release the amniotic membrane extract at the wound site after 24 hours. The in vitro tests showed that the blood cells in the vicinity of the prepared dressing had a very good tube formation after 48 hours compared to the sterile gas control sample.

DISCUSSION & CONCLUSIONS: Because of the lengthy treatment times, delays in healing of chronic wounds, they have an impact on quality of life and on costs. Today, silicone wound dressings are widely used in the repair of chronic wounds. It can be concluded that skin tissue engineering using GelMA to the silicone surface and amniotic membrane extract can speed up the healing of wound while preventing exudate buildup and the formation of scar tissue.

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Keywords: Wound healing,

PP-465

Impact of microstructure on cell behavior and tissue mechanics in collagen and dermal decellularized extra-cellular matrices

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INTRODUCTION: Skin models are used for many applications such as research and development or grafting. However, most of them lack a proper microenvironment leading to poor mechanical properties and inaccurate extra-cellular matrix composition and organization, leading to limited predictivity and weakness of skin substitutes after grafting. We focused on mechanical properties, extra-cellular matrix organization and cell interactions in human skin samples reconstructed with pure collagen or dermal decellularized extra-cellular matrices and compared them to native human skin.

METHODS: Normal human fibroblasts or keratinocytes from skin plastic surgery wastes have been printed using inkjet methods or manually seeded. To reproduce the dermis part porcine dermal skin decellularized extra-cellular matrix (S-dECM) was prepared.

RESULTS: Full-thickness S-dECM samples presented stiffness two times higher than collagen gel and similar to ex vivo human skin, and proved for the first time that keratinocytes also impact dermal mechanical properties. This was correlated with larger fibers in S-dECM matrices compared to collagen samples and with a differential expression of F-actin, vinculin and tenascin C between S-dECM and collagen samples.

DISCUSSION & CONCLUSIONS: This study shows, for the first time, the importance of a complex and rich microenvironment on cell behaviors, matrix macro- and micro-organization and mechanical properties. The increased composition and organization complexity of dermal skin decellularized extra-cellular matrix populated with differentiated cells produces in vitro skin models closer to native human skin physiology. This is clear proof of the microenvironment's impact on cell behaviors and mechanical properties.

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Keywords: Skin, Microenvironment and niche engineering

PP-466

Bioprinting of functional cardiac tissue using a collagen-based bioink

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INTRODUCTION: Engineered cardiac tissues are valuable tools to study disease and elucidate drug responses, and even show promise as therapeutic agents to support an injured heart. Such tissues are most commonly produced by casting cells embedded in a biomaterial matrix into molds to consolidate. More recently, 3D bioprinting has been used to generate cardiac tissues. This technology allows for the generation of tissues with more complex geometries, inherent hierarchy and even anisotropy. The cardiac tissues produced by 3D bioprinting to date, however, showed limited functionality or required support structures for stability.

METHODS: Here, a bioprinting approach is employed which enables the generation of functional cardiac tissues from hiPSC-derived cardiomyocytes, without the need for support structures. To do so, hiPSC-derived cardiomyocytes are suspended in a collagen-I/hyaluronic acid bioink, printed into a support bath comprised of gelating/gum arabic microparticles, and extracted after bioink gelation. In this manner, constructs with complex geometries, as well as two-component constructs were generated.

RESULTS: Bioprinted ring-shaped cardiac tissues (5 mm diameter, 1 mm height) displayed synchronous contractions as early as 3 days after fabrication, which persisted over the course of cultivation for 30 days. In addition, beating frequencies could be modulated by stimulation with adrenergic stimuli or calcium blockers. Of note, ring-shaped cardiac tissues were capable of deflecting flexible silicone pillars. HiPSC-derived cardiomyocytes within printed constructs displayed elongated morphology and a typical striated pattern of sarcomeric proteins. To demonstrate the potential of this approach to produce cardiac tissues with more complex geometries, balloon-shaped, simplified models of single ventricles (8 mm diameter, 14 mm height) were printed. Similar to ring-shaped tissues, ventricles displayed synchronous contractions after 3 days, and could be maintained in culture for up to 3 months. Concurrently, ventricles were responsive to pharmacological stimulation, and cardiomyocytes displayed striated sarcomeric pattern. To retain an open lumen, bioprinted ventricles were connected to silicone tubing and cultured upright.

DISCUSSION & CONCLUSIONS: 3D bioprinting is a promising technology to improve current cardiac tissue models. The approach described here enables fabrication of functional tissues in a direct manner, without the need for support structures. In this manner, simplified models of heart ventricles could be generated, which display synchronous contractions and can be cultivated for several months.

Keywords: 3D printing and bioprinting, Biofabrication

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Laser-Induced Graphene Anisotropic Substrates for Cardiac Tissue Engineering

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INTRODUCTION: Mortality linked to cardiovascular diseases remains a health challenge.

Cardiomyocyte-based tissue engineering is frequently employed to develop effective therapies by creating disease in vitro models. However, the cardiomyocytes and tissues generated are recurrently immature, limiting their widespread use. Nevertheless, cardiac tissue engineering has been a powerful tool for building representative cardiac tissues. Conductive materials, such as graphene, can stimulate and be used to mature naive cardiomyocytes [1]. However, the conventional graphene generation method presents downsides: complexity and low-yield production. Conversely, laser-induced graphene (LIG) [2], which can reduce conventional graphene production costs, is eco-friendlier and sustainable while still showing efficient conductivity and biocompatibility. Hence, this work aims to bioengineer a LIG-based substrate for cell culture. It is hypothesized that the LIG conductive material can stimulate the cardiomyocytes and enhance their maturation, combining conductivity with LIG-derived anisotropy. These in vitro cardiomyocyte-based systems will mimic the cardiac niche and be generated for cardiac therapy development based on more representative cardiac tissues.

METHODS: The LIG was generated using a CO₂ laser, with Parylene-C as the precursor, and the material generated was thoroughly characterized as previously described [3]. Cell culture was performed first with stem cells and followed by embryoid bodies, and both cell cultures were characterized for viability, metabolic activity, morphological structure, and immunofluorescence. All experiments were performed independently and with a minimum of three biological replicates. In addition, graphene pristine, collagen, and gelatin were assessed as controls.

RESULTS: Both cell types were viable within the LIG and metabolically active during the culture period. The beating cardiomyocytes derived from the embryoid bodies were also differentiated in the conductive and anisotropic LIG. These cardiomyocytes were viable and metabolically active after the culture period. In addition, cardiac-specific proteins and structures were detected after immunofluorescence and electron-microscopy characterization.

DISCUSSION & CONCLUSIONS: For the first time, stem cells and embryoid bodies were in culture in LIG-based substrates, with a positive indication that this material is biocompatible, especially with cardiomyocytes from embryoid bodies. This sustainable LIG material shows tremendous potential for cardiac tissue engineering applications.

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Keywords: Cardiovascular, Nanomaterials (inc graphene)

PP-468

Systemic delivery of immune cells improves the cardiac repair outcome following myocardial infarction

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INTRODUCTION: Each year 15 million people suffer from a myocardial infarction resulting in one of the leading causes of death worldwide. Owing to the fact that the adult heart lacks a regenerative capacity, the ischemic cardiac muscle is replaced by scar tissue. While the mechanisms involved in fibrotic tissue formation are still elusive, the immune system is known to play a critical role.

Therefore, modulating the immune response after cardiac injury is a promising strategy to improve heart function after myocardial infarction.

METHODS: Using a permanent left coronary artery ligation mouse model, we assessed the cardiac repair after myocardial infarction, in two conditions. The first condition involved depletion of a specific immune cell subtype with diphtheria toxin, using a genetically engineered model. On the other hand, the second condition involved the adoptive transfer of these cells in a clinically relevant manner, one day post ischemia, in wild type mice. The reparative cardiac capacity was assessed with echocardiography, an angiogenesis assay, and Mason's trichrome staining to assess the fibrotic area. To investigate the mechanisms of cardiac repair, we performed an EdU proliferation assay and a TUNEL survival assay on cardiomyocytes. Moreover, we performed flow cytometry and RNA sequencing on various immune cell subsets isolated from the infarcts to determine changes in the inflammatory response.

RESULTS: Upon depletion of this particular immune cell subset, there was a significant increase in infarct size and a reduced left ventricular contractility (reduced ejection fraction and increased end diastolic volume), as evidenced with echocardiography. On the other hand, the adoptive transfer of the immune cell subset improved cardiac repair by reducing fibrosis and increasing the left ventricular contractility. This functional improvement was accompanied by enhanced angiogenesis in the infarct area, reduced cardiomyocyte cell death and moderately increased cardiomyocyte proliferation. In addition, the immune cells delivered by adoptive transfer accumulated at the site of cardiac injury and in secondary lymphoid organs post myocardial infarction. Mechanistically the pro-repair effect of this therapy was attributed to the differences in accumulation and the inflammatory phenotype of T cells and macrophages in the infarcts.

DISCUSSION & CONCLUSIONS: Taken together, we found that the delivery of this immune cell subset post ischemia diminished scar tissue formation by acting on multiple target populations and cellular processes, resulting in long-term improvement of cardiac function. This study demonstrates the potential of using this immune cell subset as a therapy for patients with myocardial infarction and potential heart failure.

Keywords: Cardiovascular, Cell therapy

PP-469

Crosslinked extracellular matrix biomaterials functionalized with 2D-nanomaterial MXenes for cardiac tissue engineering drive iPSC-derived cardiomyocyte maturation

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INTRODUCTION: Electroconductive materials are an emerging class of biomaterials used to mimic the electrical features of native tissues such as muscle, neural, and bone [3-4] since it has been demonstrated that they promote the proliferation and differentiation behaviour of electrical stimuli-responsive cells, such as neurons and cardiomyocytes (CMs).[1-3]

METHODS: In this work, conductive biohybrid platforms were engineered by blending collagen type I and 2D MXene (Ti₃C₂T_x) to harness the biofunctionality of the protein component and the increased stiffness and enhanced electrical conductivity (matching and even surpassing native tissues) that two-dimensional titanium carbide provides.

RESULTS: These MXene platforms were highly biocompatible and resulted in increased proliferation and cell spreading when seeded with fibroblasts. Conversely, they limited bacterial attachment (*Staphylococcus aureus*) and proliferation. When neonatal rat cardiomyocytes (nrCMs) were cultured on the substrates increased spreading and viability up to day 7 was studied when compared to control collagen substrates. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were seeded and stimulated using electric-field generation in a custom-made bioreactor. The combination of an electroconductive substrate with external electrical field enhanced cell growth, and significantly increased cx43 expression.

DISCUSSION & CONCLUSIONS: These findings are promising in the development of highly biocompatible and conductive biohybrid platforms that may be tailored applied to a variety of biological applications requiring electrical conductivity. Furthermore, this work has demonstrated the excellent features of MXene and its prospective usage in the future as a component with natural polymers. Indeed it could be implanted into electrically conductive tissues to serve as an electrically conductive bridge or scaffold, as well as an in vitro platform to grow electrically sensitive cells outside the body.

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Keywords: Nanomaterials (inc graphene), Induced pluripotent stem cells

PP-470

Developing a microfluidic model to investigate the crosstalk between *Porphyromonas gingivalis* and vascular endothelial cells

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INTRODUCTION: Periodontitis, a chronic oral inflammatory disease, associated with *Porphyromonas gingivalis*, has been linked with increased atherosclerotic complications, with bacteraemia postulated to lead to activation of platelets and vascular endothelial cells (VECs). However, the mechanism by which infection can impact platelet-VEC interactions is unclear. To model these periodontopathogen-host cell interactions, an in-vitro model of vasculature is required where the interplay between *P. gingivalis* and VECs under physiological environments can be investigated.

METHODS: Using the ibidiTM microfluidic system, a vascular model was developed, with seeding density, shear stress and flow rates optimised to enable adherence and alignment of human umbilical vein endothelial cells (HUVECs). *P. gingivalis* was perfused over aligned HUVECs, and bacterial invasion under static and flow conditions following 4h and 24h infection was determined by immunofluorescence. To establish a 3D model, type I collagen, fibrin, and thrombin gels were first utilised to form 3D culture of fibroblasts and human aortic smooth muscle cells (HASMCs) individually for 7-days with viability assessed using calcein-AM/propidium iodide. 3D co-culture of fibroblasts and HASMCs (50:50) were established and maintained for 2-weeks to create an intima-like matrix. HUVECs were then seeded on the surface of the 3D co-culture system and further cultivated for 4-weeks. Histology and immunohistochemistry will be conducted to confirm the production of α -smooth muscle actin and presence and type of extracellular matrix (ECM). The 3D constructs will be cultured under flow using the ibidiTM microfluidic system to investigate interactions with the periodontal pathogens.

RESULTS: Optimal cell adherence of HUVECs was observed with type IV collagen coated ibiTreatTM μ -slides 0.4 mm. Cell realignment occurred after 2-days under flow at 25 dyn/cm² compared to static conditions. Immunofluorescence confirmed *P. gingivalis* infection of HUVECs with an increased number of cells infected following 24h versus 4h. Fibroblasts and HASMCs each cultured in type I collagen-fibrin-thrombin gels were viable following 7-days of culture. The generated 3D co-culture system is currently being validated.

DISCUSSION & CONCLUSIONS: HUVECs re-aligned in response to in-vivo simulated shear stress, as would be expected in an in-vivo artery. Ongoing work will determine the effects of *P. gingivalis* invasion of the 3D co-culture system under perfusion. Our findings will help better understand mechanisms by which periodontal pathogens might be contributing to atherosclerosis and could help inform patient treatment.

Keywords: Cardiovascular, Disease models

PP-471

Induce and control the phenotypic switch in ipsc-derived vascular smooth muscle cells in a 3d hydrogel

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INTRODUCTION: Vascular smooth muscle cells (VSMCs) play a major role in arteriole vasomotion control and can be classified in two pheno states: Contractile VSMCs, A non-proliferative, non-migrating cell phenotype responsible for the contraction and relaxation control of the blood vessel. Synthetic VSMCs, a proliferative, migrating cell phenotype that contributes to extracellular matrix buildup to allow growth of neo-vessels. In order to prevent undesired maladaptive switching in disease conditions and to be able to harness this mechanism for vascular generative purposes, we need to improve our understanding of the biological and mechanical cues for this phenotypic switch. It has been described that it's possible to induce the switch in aortic VSMCs (aVSMCs) using growth factors and mechanical cues including cyclic stretch and material stiffness in a 2D cell culture(1).

METHODS: We hypothesize that human iPSCs vascular Organoid Derived smooth Muscle Cells (ODMCs) can switch from a synthetic to a contractile phenotype by different combinations of biological and mechanical cues. Isolation and culture of ODMCs have been described in previous research published by our group(2). The phenotypic switch was induced in a 2D environment using the growth factors TGF- β and PDGF. To evaluate the ODMCs response to ECM stiffness and cyclic strain, contractile and synthetic ODMCs encased in 3D GelMa hydrogel with different stiffness are cultured in static conditions and stretched in a FlexCell set-up.

RESULTS: 2D growth-factor experiments showed that both ODMCs and VSMCs stop proliferating and changed morphologically to a spindle-shaped cell type after treatment with TGF- β . Contractile genes are upregulated after growth-factor treatment in both cell types. Cell culture in a 3D GelMa-based environment showed that a stiffer hydrogel caused significant upregulation of contractile markers on gene-expression and protein levels. Preliminary results indicate that dynamic stimulation also induces a more contractile phenotype.

DISCUSSION & CONCLUSIONS: In conclusion, both growth factors in 2D and the stiffness of the 3D environment influence the phenotypic switch of ODMCs. Ongoing research on the adaptation of ODMCs to cyclic strain in different 3D hydrogel conditions will provide further crucial insight in the contribution of hydrogel stiffness to the strain response of ODMCs.

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Keywords: Vascular systems / vascularisation and heart, Induced pluripotent stem cells

PP-472

Mimicking the glomerular endothelium; induce and control fenestrations in iPSC-derived endothelial cells

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INTRODUCTION: Chronic kidney disease (CKD), which leads to progressive and irreversible reduction in renal function and increase in cardiovascular disease risk, is a growing global public health concern. CKD is associated with a decline in filtration. Critical for this process is reduction in function of glomerular units of the nephrons, which contain capillaries with an endothelium that is characterized by the presence of fenestrae on the order of 70–100 nm in diameter to facilitate filtration. To develop in vitro (disease) models that more closely mimic human glomerular capillaries, a better understanding of the development fenestrations is essential.

METHODS: Here, we are working towards a human, potentially patient-specific disease in vitro model for fenestrated endothelium using induced pluripotent stem cells (iPSC) vascular Organoid-Derived Endothelial Cells (ODECs). Isolation and culture of ODECs has been described in previous research published by our group (Meijer et al.). The ODECs are not fenestrated after initial differentiation and isolation, as compared to Human Glomerular Endothelial Cells (HRGECs). Introduction of fenestrations by adding growth factors VEGF-a and PMA was evaluated, and size and number control of fenestrations by adding Cytochalasin B and Diamide was studied. The effect of shear stress on the expression of Plasmalemma Vesicle Associated Protein (PLVAP), the protein that forms the stomatal and fenestral diaphragms was evaluated by flowing of the monolayer in an ibidi cell culture system.

RESULTS: Gene-expression analysis showed that both VEGF-a and PMA induce the formation of fenestrations in ODECs, indicated by upregulation of PLVAP. This was confirmed by SEM imaging where the number and diameter of the fenestrations was comparable to fenestrations in HRGECs. SEM analysis also confirmed that the number fenestrae increases after Cytochalasin-B treatment. Diamide treatment caused a significant decrease in fenestration diameter, as expected and described in previous literature. These findings were supported by gene-expression analysis and western-blot data. Preliminary results show that the introduction of shear stress on the monolayers also increases the expression of PLVAP.

DISCUSSION & CONCLUSIONS: In conclusion, by using growth factor stimulation and Cytochalasin-B treatment, we were able to induce and control fenestrations in non-fenestrated iPSC-derived endothelial cells. These findings can aid in the creation of an in vitro (patient-specific) glomerular disease model and drug-screening platform.

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Keywords: Kidney, Vascular systems / vascularisation and heart

PP-473

Developing Low Temperature Plasma (LTP) Customizable Scaffolds for Engineered Blood Vessels

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INTRODUCTION: By the year 2030 around 40.5 % of the US population is expected to be affected by some form of cardiovascular diseases (CVD) [1,2]. Bypass surgery, using the autologous vein has been one of the most effective treatments for CVD. More recently tissue engineering is gaining lot of usage for engineered vascular grafts to synthesize blood vessels Vascular grafts made of Dacron and ePTFE, are in use however, these does not work well for small diameter grafts (<6 mm) due to intimal hyperplasia and thrombosis [3-4]. In our study we plan to improve the endothelialization of intimal surface of graft by using low temperature plasma (LTP) to increase the cell attachment/ viability and proliferation.

METHODS: PTFE was treated with LTP to improve the endothelialization of graft surface. Scaffolds were also modified with gelatin and collagen. Human umbilical vein endothelial cells (HUVEC) were plated on the developed scaffolds and cell proliferation was determined by the MTT assay and by microscopy. mRNA expressions levels of different cell markers were investigated using quantitative real-time PCR (qPCR).

RESULTS: XPS confirmed the introduction of oxygenated functionalities from LTP. HUVEC cells showed 80% seeding efficiency on the scaffold. Microscopic and MTT assays indicated increase in cell viability in LTP treated scaffolds more after protein treatment. Gene expression studies shows enhanced expression of cell adhesion marker Integrin- α 5 gene after LTP treatment.

DISCUSSION & CONCLUSIONS: LTP treated scaffolds exhibited better cell proliferation and viability compared to untreated scaffolds. Protein treatment of scaffold increased cell proliferation.

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Keywords: Vascular systems / vascularisation and heart,

PP-474

Analysis of potential danger signals on PCL-hyaluronic acid-based tendon biomimetic scaffolds with a TLR-reporter cell platform

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INTRODUCTION: Tendon biomimetic scaffolds are a promising, therapeutical approach for tendinopathies¹. However, in order to be clinically effective, it is crucial that scaffolds induce a minimal immunological response when being recognized as a foreign body.

Toll-like receptors (TLRs) are one of the main receptors involved in the early stages of the innate immune response and the subsequent activation of the adaptive immune response². Up to date, little is known about their interaction with polycaprolactone (PCL)-based scaffolds.

METHODS: In this study, we have applied Jurkat cells expressing human TLR (2/1, 2/1/6, 6, 4) on electrospun PCL-hyaluronic acid (HA)-based scaffolds. In these cells, the triggering of the receptors leads to the NF- κ B promoter activation, resulting in the expression of the fluorescent molecule GFP. In parallel, propidium iodide was used in order to assess the viability of the cells. The fluorescence of GFP and propidium iodide were measured by flow cytometry after 24, 48 and 72 hours of contact with the scaffolds. For the latter, different concentrations of PCL (12 or 20%) were used to obtain different fiber diameters, in combination with different HA concentrations (0 or 0,5 or 0,75%) to improve their hydrophilic properties and cellular performance. Besides, different fiber spatial distributions were generated (random and aligned) and tested.

RESULTS: Our results show that the reporter cells viability is not affected by the tested biomaterials. Concerning the TLR recognition, only TLR 2/1 and TLR 4 were slightly triggered by the specimens composed by 20% PCL, independently of their fiber spatial distribution. Additionally, some of the scaffold compositions show a lower activation signal than the background, suggesting a possible inhibitory effect of those specimens.

DISCUSSION & CONCLUSIONS: In conclusion, TLR reporter cells have shown to be a useful and effective tool to study the interaction between scaffolds and danger-sensing receptors.

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Keywords: Biomaterials, Immunity / immunomodulation / macrophage

PP-475

Dendritic cells secrete epithelial growth factor receptor-ligands upon stimulation with oxidized phospholipids

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INTRODUCTION: Dendritic cells (DC) are the most potent antigen-presenting cells and are essential in the first line defence against invading pathogens and for the regulation of immune responses in various inflammatory diseases. By connecting innate and adaptive immune responses DCs are capable in increasing protective immune responses as well as negatively regulating the course of T cell immunity. Oxidized phospholipids (ox-PLs), which are generated during inflammatory reactions by ROS- mediated oxidation, are known to dysregulate the functional differentiation of DCs leading to a reduce capacity to induce proliferation of T cells.

METHODS: We firstly differentiate dendritic cells from primary monocytes Then, we analyzed whether the oxidized phospholipid OxPAPC which is generated by ROS- mediated oxidation could shift the expression pattern of DC.

RESULTS: We found that OxPAPC-treated DC up-regulate the EGFR-ligands (EGFR- L) amphiregulin, epiregulin, heparin-binding-EGF and TGF- α . Stimulation of DCs with TLR- ligands Pam3CSK4, Poly I:C or LPS did not induce the production of EGFR-Ls. However, augmented amounts of all four EGFR-L were synergistically induced by a combination of ox- PLs with TLR-ligands. We also uncovered that Nurr1 and the cAMP pathway are involved in the signalling mechanisms responsible for EGFR-L expression in DCs. Finally, DCs or supernatants of DCs stimulated with ox-PLs were able to stimulate cell proliferation in an EGFR-dependent manner.

DISCUSSION & CONCLUSIONS: Here we demonstrate that the ox-PL OxPAPC shifts the protein expression pattern of DC towards the ligands for the epidermal growth factor receptor (EGFR). DCs gain the function to stimulate the growth and differentiation of tissue cells upon contact with inflammation-born ox-PLs, a process which might be involved in tissue regeneration.

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Keywords: Immunity / immunomodulation / macrophage, Other

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The secretome of mesenchymal stromal cells modulates B cells from patients with Rheumatoid Arthritis

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INTRODUCTION: Mesenchymal stromal cells (MSC) from the amniotic membrane of human term placenta (hAMSC) and their secretome (conditioned medium, CM-hAMSC) have enormous potential for their use in regenerative medicine mainly due to their immunomodulatory properties. Many in vitro studies showed that hAMSC and CM-hAMSC modulate the proliferation, maturation, and cytokine secretion profile of cells of the innate and adaptive immune systems, and support the expansion of regulatory immune cells. Furthermore, in vivo studies using hAMSC and CM-hAMSC report beneficial effects in different animal models of diseases associated with inflammatory conditions and degenerative processes, such as lung and liver fibrosis, myocardial ischemia, traumatic brain injury, wound healing in diabetic mice, and autoimmune diseases, such as rheumatoid arthritis. Of note, we have recently demonstrated in vitro that CM-hAMSC strongly acts also on B cells, suppressing their proliferation and reducing their terminal differentiation into plasma cells.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovial tissue inflammation leading ultimately to irreversible joint destruction. Given that B cells have a prominent role in RA pathogenesis and considering the therapeutic benefit and safety of depleting B cells detected in humans, the possibility to modulate B cell activation and functions by exploiting hAMSC immunomodulatory properties could be a novel therapeutic strategy. Thus, the aim of this study was to investigate the possible effect of CM-hAMSC on peripheral B lymphocytes from patients with active RA.

METHODS: Peripheral blood mononuclear cells isolated from active RA patients were stimulated in vitro with CpG-ODN, and cultured in presence or absence of CM-hAMSC. After 6 days of culture, flow cytometry analysis was performed for the evaluation of B-cell proliferation and differentiation.

RESULTS: Collectively, our results clearly demonstrate that the hAMSC secretome reduces the CpG-triggered proliferation of CD19+ B cells from RA, similarly to what we previously observed with B cells

from healthy donors. We also demonstrate that CM-hAMSC treatment mostly affected antibody-secreting cells (ASC, CD27^{high}CD38^{high}), not only reducing their formation but also blocking their maturation into terminally differentiated plasma cells (CD27^{high}CD38^{high}CD138⁺).

DISCUSSION & CONCLUSIONS: In conclusion, we demonstrate that the bioactive factors released by hAMSC strongly interfere with the proliferation and terminal differentiation of peripheral blood B cells of patients affected by RA.

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Keywords: Multipotent (mesenchymal) stem cells, Musculoskeletal (inc ligament / tendon / muscle / etc)

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Modulating macrophage polarization *in vitro* with ultra-short peptide-polymer bioconjugates for tissue engineering applications

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INTRODUCTION: Macrophages are prominent immune cells involved in inflammatory responses. Interestingly, recent studies have found the role of macrophages in tissue regeneration mainly by the secretion of cytokines, chemokines, and growth factors which aid in wound healing, tissue repair, and angiogenesis. In the presence of biomaterials, the response of macrophages plays a crucial part in implant rejection or integration, depending on the material-mediated alteration in macrophage phenotype. Biomaterial-macrophage interactions mostly polarize the macrophages into pro-inflammatory M1 phenotype or anti-inflammatory M2 phenotype. This polarization decides the fate of the biomaterial towards chronic inflammation or regeneration. Hence, here we studied the biomaterial-mediated polarization of macrophages and its impact on wound healing, stem cell differentiation, and angiogenesis.

METHODS: Ultrashort peptides were synthesised using solution phase synthesis protocol and characterised using NMR, HRMS, SEM, TEM and FTIR. Polymer-peptide bioconjugate was synthesised using EDC coupling reaction and subjected to material characterisation via SEM, NMR, HRMS and FTIR. Macrophage polarisation on the bioconjugates was evaluated using Griess assay, arginase assay, RTqPCR, immunofluorescence, ELISA and morphological changes. Further the wound healing and angiogenesis capability of macrophages incorporated biomaterial was determined by direct and indirect co-culture methods.

RESULTS: Peptide-incorporated hydrogels exhibited excellent biocompatibility and biodegradability. Macrophage polarization studies revealed the polarisation of macrophages towards the anti-inflammatory phenotype to varying degrees based on the biomaterials. Wound healing assay showed the enhanced closure of the wound in the test group Tryptophan and tyrosine ultrashort peptide incorporated chitosan hydrogel promoted the polarization of monocytes towards an anti-inflammatory, regenerative M2 phenotype.

DISCUSSION & CONCLUSIONS: Bioconjugates containing polymers and ultrashort aromatic peptides were successfully synthesised and characterised. Upon evaluation of macrophage polarisation, modulation towards anti-inflammatory phenotype was observed in the test group which also showed excellent wound healing capability.

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Keywords: Wound healing, Immunity / immunomodulation / macrophage

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Combined biolistic and cell penetrating systems for the development of DNA vaccines

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INTRODUCTION: Physical-based gene delivery via biolistic methods involves precipitation of DNA onto microparticles (MPs) and its direct transfection into cells in exposed tissue (e.g., skin) by high velocity acceleration, an example being the Helios gene gun system with gold MPs. The Glycosaminoglycan (GAG)-binding enhanced transduction (GET) system exploits novel fusion peptides consisting of cell-binding, nucleic acid condensing, and cell-penetrating domains, which enable enhanced transfection across multiple cell types with cell exposure. In this study, we explored combining non-physical (GET) and physical (gene gun) DNA delivery systems. We hypothesized that the combination of two different mechanisms will generate enhanced distribution of the formulations, with more effective uptake when not directly delivered within the targeted tissue.

METHODS: Initial transfection studies explored various formulations to determine the optimal GET-gold-DNA ratio. The next stage involved incorporating the GET-gold formulation into fireable gene gun bullets. Subsequent experiments explored the physicochemical characterization, optimization of bullet contents and transfection experiments in vitro on cell monolayers, engineered tissue and in vivo on mice.

RESULTS: Transfection studies showed that GET-gold formulation using DNA-protein charge ratio of 50 and 0.5 mg gold/ μ g DNA provided a significant increase (p -value < 0.01) in reporter gene expression in DC2.4, a mouse dendritic cell line. We incorporated this into gene gun bullets by complexing and compacting DNA with the GET-gold, forming a dry formulation for firing by freeze drying. Flushed GET bullet contents retained their ability to mediate transfection which was nearly 20-fold greater than that of standard spermidine bullets. Lastly, fired GET bullets in vitro (in cells and collagen gels) and in vivo (mice) showed increased reporter gene transfection compared to untreated cells, whilst maintaining cell viability in vitro and having no obvious toxicity in vivo.

DISCUSSION & CONCLUSIONS: In conclusion, the non-physical and physical combination of GET-gold-DNA using gene gun showed potential as an alternative DNA delivery method. We aim to test this system in the future in in vivo DNA vaccine studies.

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Curcumin loaded zinc mesoporous glass nanoparticles for infection treatment and bone regeneration

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INTRODUCTION: Nanotechnology-based approaches are emerging as promising strategies to regenerate bone and fight infections [1]. A nanodevice based on mesoporous SiO₂-CaO-P₂O₅ glass nanoparticles enriched with Zn²⁺ and loaded with curcumin was designed. Thus, several types of nanoparticles are being investigated, including those based on mesoporous bioactive glasses (MGNs) that exhibit outstanding structural and textural properties, as well as the capability to be loaded with biologically active substances in their mesopores.

METHODS: The MGNs were synthesised using the sol-gel chemistry-based procedure described by Liang et al [2]. MGN1, with composition 60SiO₂-4P₂O₅-(36-x)CaO, and MGN2, with composition 79.5SiO₂-2.5P₂O₅-(18-x)CaO, being x = 0, 2.5, 4 mol-% of ZnO. Consequently, six nanomaterials were obtained: MGN1/2, MGN1/2-2.5Zn and MGN1/2-4Zn. MGNs curcumin loading and release were evaluated. In vitro cytocompatibility, Flow cytometry, Mineralization assays and Real time PCR measurements with MC3T3-E1 and MSCs cell lines. In vitro bacterial assays: planktonic growth inhibition, biofilm degradation and cells and bacteria co-culture assays were evaluated.

RESULTS: Radial Zink-MGNs were synthesised with 100 nm, bimodal diameters of 2.5 nm and 6-8 nm, and 600-800 m²/g of surface areas. NMR and EDX analyses demonstrated that the incorporation of phosphorous in the nanoparticles is more effective with increase of zinc content.

MGNs presented cytocompatibility with 125 and 150 µg/mL MGNs concentrations for both MC3T3-E1 preosteoblasts and MSCs with and without curcumin.

Antibacterial assays showed that zinc enhanced the bactericidal effect of curcumin against *S. aureus* in both planktonic and sessile states. The MIC and MBC values of curcumin for bacteria were significantly reduced with the zinc/curcumin combination. MGNs were able to degrade a biofilm preformed of *S. aureus*, demonstrating its suitability as a bactericide agent by up to 90% compared to control.

Results obtained in co-cultures, showed that these MGN-ZNCur nanosystems could combat antibiotic resistance in the treatment of bacterial infection and bone regeneration.

DISCUSSION & CONCLUSIONS: Synergistic effect of Zn-MGNs upgraded with zinc and curcumin that reduce significantly the bacterial growing in planktonic as well as degrading a pre-formed biofilm was demonstrated. They are cytocompatible with preosteoblasts and MSCs cells. Based on these results, MBG nanocarrier represents a solid alternative in the treatment of acute and chronic infections in bone tissue, while avoiding the currently important problem of bacterial resistance.

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Keywords: Infection, Nanomaterials (inc graphene)

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Immobilization of Toll like Receptor 2 on Mesoporous Silica Nanosystems to Combat Bacterial Infection

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INTRODUCTION: Currently, antibiotic resistance is a serious human global health hazard [1]. The shortage of new antibiotics in the phases of clinical development makes this devastating scenario more alarming. Thus, new antibacterial strategies are urgently needed to overcome this issue. Nanotechnology has landed in this arena by providing nanocarriers that deliver their antibiotic cargo at the target place [2]. Owing to their high loading capacity, easy-to-functionalize surface, stability and biocompatibility, mesoporous silica nanoparticles (MSNs) become promising nanocarriers for drug delivery [3]. Herein, we have developed a new antimicrobial nanosystem by immobilization of toll like receptor 2 (TLR2) on the external surface of levofloxacin (Levo) loaded MSNs. The ability of TLR2 to identify, guide and internalize the nanosystem into Gram-positive bacteria cell walls prompts antibacterial efficiency.

METHODS: MSNs were functionalized with aminopropyl groups (MSN-NH₂), then loaded with Levo by an impregnation method (MSN-NH₂-Levo) and finally decorated with TLR2 by using glutaraldehyde in a phosphate buffer saline solution (MSN-Levo-TLR2). The different materials were characterized by TEM, EDS, DLS, ELS, FTIR, TGA and elemental analysis. "In vial" Levo release tests and in vitro studies against Gram-positive *S. aureus* bacteria were carried out.

RESULTS: Antibiotic loaded MSNs externally functionalized with TLR2 receptor have been successfully synthesized. In vitro Levo release from such nanosystems shows a sustained release of the antimicrobial, which is more pronounced in the presence of the TLR2 receptor, extending the administration for a longer time compared to non-functionalised nanosystems. These results could be explained on the basis that the receptor prevents to a certain degree the outflow of the antibiotic loaded in the mesopores of MSNs. In vitro microbiological assays show that the TLR2 incorporation enhances the antibacterial capacity against Gram-positive bacteria, due to its ability to recognise and target the bacterial wall, compared to the non-functionalized nanosystem.

DISCUSSION & CONCLUSIONS: The incorporation of TLR2 onto the MSNs surface promises a very promising future in the treatment and diagnosis of gram-positive bacterial infections and for future personalized therapies.

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Electrophoretic Deposition of Copper-Doped Coatings for Soft Tissue Integration

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INTRODUCTION: Dental implants are widely used in (partially) edentulous patients. However, peri-implant soft tissues are less vascularized and cellularized compared to those around natural teeth, which increases the risk of bacterial invasion and ultimately implant loss [1]. A particularly promising strategy to improve soft tissue integration is to incorporate therapeutic factors on implant surfaces. Copper ion or Cu(II) is a promising therapeutic agent with angiogenic and antibacterial properties beneficial for wound healing [2]. The amount of released Cu(II) must be precisely controlled within a therapeutic window. To this end, we introduced a simple and cost-effective to tune Cu(II) release through an electrophoretic deposition (EPD) technique and evaluate the biological characterizations of functional coatings in vitro [3].

METHODS: Cu(II) was firstly doped into bioactive glass (BG) nanoparticles (Cu-BG). Subsequently, chitosan coatings containing BG, Cu-BG, or a mixture thereof (1:1 w/w ratio) were deposited on commercial pure Ti (cpTi) discs by EPD. Coating topographies were observed using scanning electron microscopy. Ion release profiles were measured by inductively coupled plasma atomic emission spectrometry. To assess cytotoxicity, human gingival fibroblasts (HGFs) were seeded on the disks and cultured for up to 7 days using Cell Counting Kit (CCK-8) assay. Angiogenic potential was evaluated by exposing human umbilical vein endothelial cells (HUVECs) to the extracts of the coatings to measure their migration rate through a wound scratch test. Antibacterial tests were performed by measuring *Porphyromonas gingivalis* (P.g.) growth on coated/uncoated surfaces.

RESULTS: Homogeneous coatings were formed on cpTi disks. The released amount of Cu correlated to the content of deposited Cu-BG. HGFs survived on the composite coatings except for coatings with the highest amount of copper, which inhibited their growth. The migration rate of HUVECs was highest on coatings containing equal amounts of Cu-BG and BG. Antibacterial tests confirmed that Cu-doped coatings reduced the P.g. growth up to fivefold compared with uncoated implants.

DISCUSSION & CONCLUSIONS: By changing Cu-BG:BG ratio, we controlled Cu release kinetics without changing other physicochemical properties. An optimal amount of Cu in the coating with equal amounts of Cu-BG and BG was observed concerning their angiogenic and antibacterial efficacy.

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Keywords: Biomaterials, Wound healing

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Phosphorylation of rpS6 as a Spatial Indicator of Tissue Response to Wounding in Mammals: A Comprehensive Evaluation in the Oral Cavity and Multiple Skin Sites of *Sus domesticus*

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INTRODUCTION: The extent of tissue response to injury remains an area of limited understanding. Our findings demonstrate that after skin injury in mammals, ribosomal protein S6 (rps6) undergoes phosphorylation, resulting in the formation of a p-rpS6 activation zone around the wound site. This zone emerges within minutes after wounding and remains until healing is complete, encapsulating markers of healing such as proliferation, senescence, and angiogenesis.

METHODS: In this study, we present a comprehensive evaluation of the activation patterns observed in wounds using an in vivo porcine skin wounding model. Porcine skin was chosen for studying wound responses as it is structurally highly similar to human skin. This project compares wound-induced activation of the oral cavity and multiple skin regions of a pig (*Sus domesticus*), including skin of ear, snout, hock and feet, as well as dorsal and lateral regions.

RESULTS: Our results revealed differences in spatial rpS6 phosphorylation patterns across these sites and prompted us to correlate spatial properties of the p-rpS6-zone with anatomical features such as thickness of epidermis, density of vasculature as well as the number of nerves and hair follicles.

DISCUSSION & CONCLUSIONS: These findings provide valuable insight into the tissue response to wounding, contributing to a deeper understanding of the healing process and its dependency on perfusion and nerve signaling. Further research in this area may lead to the development of new therapeutic strategies to improve wound healing of chronic wound patients.

Keywords: Skin,

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Global landscape of artificial skin solutions; positioning a strategy for product development.

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INTRODUCTION: Recently there has been a rapid growth in the number of commercial and pre-commercial products, moreover, there is considerable commonality between skin and other soft tissue reconstruction products. Critical considerations for evaluating products are therapeutic modality, and potential for clinical adoption geographically, determined by regulatory territory, IP and commercial distribution factors.

METHODS: We have attempted a comprehensive global survey of clinically available advanced replacement materials and associated strategies for skin and soft tissue reconstruction. Products were documented according to eight broad categories. This makes the information amenable to strategic analysis, and highlights both similarities and differences between product categories. The survey also charted market growth, commercial activity, regulatory frameworks, economic and reimbursement issues.

RESULTS: Across the different categories examined, 263 base products for advanced skin therapy have been identified, giving over 350 products in total. The geographic reach of products is hugely disparate: the largest market is in the US, followed by the EU zone. The number of products identified, and fractional distribution across the 8 modality types are: Decellularised tissue: 144 (45%); Synthetic acellular scaffolds (23%): 75; Synthetic polymers products, 35 (11%); Extracellular engineered matrix dressings: 31 (10%); Tissue engineered skin: 15 (4.6%); Cultured cells: 11 (3.3%); Growth factors and pharmaceuticals: 11 (3.3%); Disaggregated skin: 3 (0/9%).

DISCUSSION & CONCLUSIONS: One useful outcome of this landscape is the functional classification of products. Decellularised tissues dominate the landscape, by a twofold factor over synthetic acellular scaffolds, the next most numerous of modalities represented. It is notable that advanced therapy products (tissue engineered skin equivalents and cultured cells) remain minor contributors to the landscape.

The clinical context of the market expansion of artificial skin products is a worldwide increase in demand for wound treatments, attributable to increased population age and presentation of wounds from trauma and chronic pathologies, placing considerable demands on healthcare services (e.g. 5% of NHS budget). The steady growth in artificial skin market (5-7% CAGR) indicate the underlying clinical need.

Considering emerging results of clinical and health-economic assessments of various products across the modalities, clinical efficacy and outcomes emerge as limiting parameters, despite the steady market growth.

However, despite the growth in number of products within each modality, areas of functionality are identified representing still un-met needs. This analysis identifies an opportunity and may serve as a guide for development of new products with greater efficacy or cost-benefit to meet these needs.

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Keywords: Wound healing, Skin

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Dodonea viscosa Extract Priming Increases Efficacy of ASCs To Counter Thermal Stress In-Vitro and for Burn Wounds Healing In-Vivo

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INTRODUCTION: Burn injuries lead to compromised life quality and a huge burden on health care system. In this study *Dodonaea viscosa* (DV) extract was investigated for its effects against burn induced oxidative stress and inflammation in adipose derived stem cells (ASCs) in-vitro and for healing of burn wounds in-vivo in rat heat injury model.

METHODS: In this regard ASCs were pre-treated with optimized dose of methanolic extract of DV for 24hrs and subsequently exposed to in-vitro thermal injury (51°C for 10min in water bath). Heat exposed ASCs were assessed for their viability (MTT assay) and alteration in genes expression. In-vivo thermal injury model was produced by creating burn wound on dorsum of the rats with pre-heated (100°C) round copper disc (2cm diameter) for 10 seconds. Further, a construct was prepared using either DV pretreated or non-pretreated ASCs (2×10^5) with denuded amniotic membrane (AM) in a way that ASCs were attached to the stromal side of AM. This construct was transplanted to the wound bed in orientation which faced stem cells to the wound bed while basement membrane of the AM to the outer wound edges. Non-transplanted heat injured and normal non heat-injured rats with excisional wounds were kept as burn injury control and normal control respectively. Wound healing potential of all groups was assessed by gross morphology, wound closure analyses, histology, and gene expression analyses.

RESULTS: DV priming resulted in upregulation of cell survival (Bcl2/Bax ratio), antioxidant (GSR), and downregulation of apoptotic (Bax) and inflammation (IL6, Nfkb1) associated genes in ASCs in-vitro. In-vivo data depicted that DV primed ASCs induced faster wound healing (91.952%) at 24days than untreated ASCs laden AM (83.753% wound closure) and injury control (78.758%), better architecture of healed skin, normal thickness of epidermis, orderly arranged collagen fibres, and increased number of skin appendages. Gene expression analyses showed upregulation of cell survival (Bcl2/Bax ratio: 1.62 ± 0.5 folds vs 0.76 ± 0.3 folds & 0.6 ± 0.03 folds at day12), angiogenic (Vegf: 3.7 ± 0.04 vs 1.8 ± 0.01 & 1.91 ± 0.04 folds at day12), proliferation (Pcna: 1.01 ± 0.0 vs 0.45 ± 0.3 & 0.8 ± 0.03 folds, Tgf β 1: 3.08 ± 0.3 vs 1.55 ± 0.09 & 1.6 ± 0.08 folds at day24), and structural (Krt1: 2.01 ± 0.15 vs 0.15 ± 0.03 & 0.89 ± 0.16 folds, Krt15: 1.6 ± 0.01 vs 0.86 ± 0.23 & 0.30 ± 0.01 folds, Krt19: 0.91 ± 0.04 vs 0.32 ± 0.09 & 0.7 ± 0.01 folds at day24) genes in comparison to untreated ASCs laden AM transplanted and heat injured rats respectively

DISCUSSION & CONCLUSIONS: In conclusion, the combination therapy of DV primed ASCs loaded AM prompts healing of thermally burnt skin with improved architecture.

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Keywords: Stem cells - general, Wound healing

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elr-based basement membranes analogues for wound healing applications

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INTRODUCTION: The dermal basement membrane serves an important role in maintaining skin integrity by regulating cell functions, as well as resisting shear forces and allowing the diffusion of bioactive molecules. Damage to the skin through diseases that affects the basement membrane is characterised by delayed healing, contractures and scarring. Here, we report the controlled production and characterisation of a biocompatible elastin-like recombinamer (ELR) based-basement membrane mimetic. González M. et al. developed a novel strategy to obtain bioinspired membranes with tunable properties. In this work, taking advantage of this methodology, an exhaustive characterization of the membranes is presented, including biological and mechanical properties.

METHODS: Two complementary ELRs were derivatised with either an azide or cyclooctyne groups and crosslinked by click chemistry. The hydrophobicity of membranes was evaluated by measurement of contact angle. Different techniques were used to assess the topography of the surfaces such as AFM, confocal microscopy or ESEM. In addition, the swelling study was carried out by gravimetric measurements. Consecutively, the ability to support the viability of skin cells was determined using HFF1 and HaCat. Cell proliferation was studied with MTS and cytotoxicity was evaluated with LDH. Cells were immunostained with different markers for studying differentiation, material-cell and cell-cell interaction and structural protein.

RESULTS: The membranes were morphologically porous and with robust mechanical properties for handling. The water in hydrogels is an important factor for the permeation of nutrients. In this regard, our ELR-membranes present a swelling of 100%. Topography studies indicated that membrane roughness can be controlled by adjustments to polymer concentration within working range for crosslinking. In this regard, it was observed that while membrane roughness decreased with increasing polymer concentration at nanoscale, the opposite effect occurs at macroscale. Crucially, viability studies showed the ELR membranes to be not cytotoxic (80% cell viability) and supported the viability of skin cells on either side and within the membrane for at least 21 days. Furthermore, analysis for α -SMA, FAK, cadherins and collagen showed support for skin cell differentiation and activities relevant for wound healing.

DISCUSSION & CONCLUSIONS: This work demonstrates the successful production of ELR-membranes with tunable physico-chemical properties. The ease of production and derivatisation, stability in culture and robust mechanical properties, as well as the promotion of cell viability and proliferation, and the expression of genes and protein characteristic in would repair make this novel ELR-based membrane an ideal candidate for use in the development of dermal substitutes for wound healing applications.

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Keywords: Biomaterials, Wound healing

PP-486

Impact of Collagen/Hyaluronic Acid for Therapeutic Multilayers Coating to Promote Wound Healing

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INTRODUCTION: Wound healing management is both time consuming and costly, as practice nurses time is very limited. The required time to treat injury leads to suboptimal administration of wound care. Therefore, there is a need for improved deployed healthcare strategies able to facilitate interventions with minimal resources, to overcome restrictions related to expertise and geographical location. In this work, a presentation of an innovative approach that uses collagen and hyaluronic acid for facilitating an efficient and faster regeneration of the skin wound. The Layer-by-layer (LbL) skin coating method is a therapeutic approach that addresses the given limitations by providing a quick, easy, and cost-effective treatment, aiming the establishment of a “re-distributed” care pathway.

METHODS: Development of a new protocol for a LBL therapeutic spray coating on polymeric pre-charged substrate to build-up bilayers of hyaluronic acid (HA) and Collagen Type I assembly (COLI). The polymeric membrane is a copolymer composite made of caprolactone (8-11%), tri-methylene carbonate (12-16%), and lactide (73-80%) (2). The study is to analyse the physical-chemical properties of the multi-layered samples using zeta potential (ZP), Fourier transform infrared spectroscopy (FTIR), and quantification of the biomolecules within the sprayed films using fluorescence microscopy. Then, an in-vitro evaluation of the multi-layered films with COLI and HA using fibroblast cells to evaluate the multilayers with the cell adhesion and adopting environment.

RESULTS: The current therapeutic approach of LbL shows a promising outcome, due to the initial cell behaviour. The polyelectrolyte films made of COLI and HA were built-up on PDLA substrate. Though, the results are collected for a short term approach which requires further and depth investigation, the results present promising data.

DISCUSSION & CONCLUSIONS: The activation of LBL assembly on the polymeric membrane presented a great potential for enabling a new care pathway that can effectively integrate with wound healing. The initial aim was to examine the possibility of translating an LBL approach to incorporate the polyelectrolyte with medically approved substrate. The current aim is to examine the possibility of translating a technique capable of delivering to a close-patient experience. The technique aims to be non-invasive and assist in accelerating wound healing.

ACKNOWLEDGEMENTS: The project was partially funded by Kuwait Foundation for the Advancement of Sciences (KFAS) under project code "CB22-63MM-01".

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Keywords: Wound healing, Skin

PP-487

Adipocytes modulate fibroblast to myofibroblast conversion: Implications for wound healing

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INTRODUCTION: The idea that adipose tissues can contribute positively to wound healing has gained significant attention over the past decade. Adipose tissues are composed of a continuum of cell types ranging from adipocyte stem cells and precursors to mature adipocytes (i.e., adipocyte lineage cells), each of which appear to play important roles in regulating extracellular matrix (ECM) remodeling. Recently, we developed a 3D in vitro system that provides an innovative approach for investigating adipocyte-fibroblast interactions in the context of wound healing and tissue remodeling^{1,2}. Using this model, we investigate the differences in fibroblast interactions with pre-adipocytes versus adipocytes.

METHODS: Pre-adipocyte spheroids were generated as previously described^{1,2} and then transferred to ultra-low adherent 24-well plates, with 5 spheroids per well. Pre-adipocyte spheroids were either maintained as pre-adipocytes in PGM-2 or differentiated into adipocytes in PDM-2 for 10 days. After 10 days, medium was removed, the spheroids were rinsed gently with 1X PBS, then 0.75 mL of DMEM containing 0.5% FBS, and 1% penicillin/streptomycin was added to each well. After two days, this medium, termed either pre-adipocyte or adipocyte conditioned medium (PCM or ACM, respectively) was collected.

To assess the ability of PCM and ACM to induce myofibroblast conversion, human dermal fibroblasts were plated at 100,000 cells/cm² in black walled 96 well plates containing 150 uL of PCM, ACM or positive control (0.5% FBS in DMEM plus 1 ng/mL TGF- β 1 and 50 mg/mL ascorbic acid). Fibroblasts were maintained for 48 hours and then the percentage of α -SMA positive cells was quantified. In parallel, pre-adipocyte and adipocyte spheroids grown on tissue culture plastic with fibroblasts for 7 days were processed, digested, and analysed using an Orbitrap Fusion LUMOS mass spectrometry (LC-MS/MS) and MaxQuant software to identify and quantify differences in the ECM protein composition.

RESULTS: We found that adipocytes, but not pre-adipocytes promote fibroblast to myofibroblast conversion characterized by expression of α -SMA and contraction. Interestingly, the conversion of fibroblasts by adipocytes occurs independent of TGF- β and creates myofibroblasts that deposit a distinct composition of matrix and collagens (Increased Col I and Col III; Decreased Col V and Col VI) compared to TGF- β induced myofibroblasts.

DISCUSSION & CONCLUSIONS: Adipocytes modulate fibroblast behavior and understanding these interactions will enable novel therapeutic approaches utilizing adipose tissues to be developed to improve the provisional matrix that is deposited, reducing scar.

ACKNOWLEDGEMENTS: Support from Fulbright-Ireland and AMBER Centre.

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Keywords: Advanced therapy medicinal products, Microenvironment and niche engineering

PP-488

The Influence of Serum Depletion on The Purity, Quality and Phospholipid Composition of Osteoblast-Derived Small Extracellular Vesicles

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INTRODUCTION: Critical-size bone defects remain a significant medical morbidity. Current treatments including autografts and allografts are limited by donor-site morbidity and graft-versus-host disease. Small Extracellular Vesicles (SEVs) are nanosized membrane-enclosed vesicles that are involved in intercellular communication and have been recognised as an attractive approach for bone repair due to their ability to regulate bone regeneration. The clinical translation of SEVs however, has been hindered by knowledge gaps regarding the impact of *in vitro* culture conditions on their composition and function. Despite extensive research on SEV cargo, current understanding of the SEV lipidome is limited. The aim of this study was to explore how different serum concentrations or using serum-free medium influences the composition of osteoblast-derived SEVs and their phospholipid profiles.

METHODS: Fetal Bovine Serum (FBS) at different starting dilutions (100%, 20% or 10%) was depleted of SEVs by ultracentrifugation at 100,000g for 18h and added to Saos-2 cells at a final concentration of 10% or 0% FBS. The viability of Saos-2 cells in SEV-depleted media or serum-free conditions was assessed via a CCK-8 assay. SEVs were extracted from Saos-2 conditioned medium collected over 24h by ultracentrifugation and characterised via nanoparticle tracking analysis, western blot, flow cytometry and mass spectrometry.

RESULTS: Saos-2 cell viability was significantly reduced ($p < 0.05$) with serum-deprived conditions but was similar across the other serum-containing conditions. All Saos-2 SEVs had sizes $< 200\text{nm}$, and were positive for TSG101, CD81 and CD63 but negative for calnexin. SEV lipid analysis revealed similarities in phosphatidylcholine (PC) species between the different SEV-depleted groups with enrichment of fatty acid chains containing 20-22 carbons. However, SEVs from serum-free cultured Saos-2 cells demonstrated different lipid profiles with enrichment of shorter (16 carbon) fatty acid chains in PC species.

DISCUSSION & CONCLUSIONS: This study showed that using SEV-depleted medium maintained the viability of cells compared to serum deprivation. Although the protein profiles of SEVs were similar, their phospholipid composition was different depending on the presence or absence of SEV-depleted FBS in culture medium. The altered lipidome in serum-starved SEVs indicates that stress conditions may affect the ability of cells to incorporate certain lipids in SEVs. The length and level of unsaturation of fatty acid chains are important as they influence membrane fluidity and stability of membrane proteins. Overall, this study emphasises the need for standardising SEV production protocols to ensure a consistent SEV lipidome and demonstrates the importance of studying the lipid composition to improve our understanding of SEV biogenesis and function.

Keywords: Extracellular vesicles, Bone and bone disorders (osteoporosis etc)

PP-489

Study of Mesenchymal Stem/Stromal Cells with Different Population Doubling Levels on Exosome Secretion Profile and Cargo Content

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INTRODUCTION: Exosomes, a type of extracellular vesicles (EVs), have a tiny size around 30-150 nm. Recently, exosomes secreted from Mesenchymal Stem/Stromal Cells (MSCs) have been widely studied and presented a great therapeutic outcome in vivo. In order to further process in the clinical evaluation state, the GMP-compliance manufacturing with completed quality development is necessary for the production of MSC-derived exosome investigational product. Based on quality by design (QbD) assessment, one of major that impacts of exosome quality is state and potential of MSCs.

METHODS: In this study, human umbilical cord-derived MSCs were isolated from three healthy donors and characterized by examining the phenotypes of MSC recognized by the International Society for Cell & Gene Therapy (ISCT) standards. MSCs were 2D-cultured in clinical-grade growth expansion culture medium with 5% human platelet lysate (hPL) and the proliferation profiles as well as number of cumulative Population Doubling Levels (cPDL) were calculated during the passage cultures. Some of MSCs in each passage were separately cultured with hPL-free medium under hypoxia condition (1% O₂) for a day after that the condition media were collected. Then, exosomes in the media were isolated and evacuated for their number, size, and their cargo contents.

RESULTS: The results showed that by increase of cPDL, MSCs have become lower secretions of exosome numbers. Moreover, the contents of several growth factors and micro-RNAs were markedly variant especially decrease of FGF, VEGF, miR-92a, and miR-23b, which related to regenerative functions.

DISCUSSION & CONCLUSIONS: Therefore, these results suggest the limitation and available range of cPDL in MSCs that can be used for guideline of cell manufacturing with acceptable quality. This study supports the fundamental information of MSCs that can be applied for design of MSC-derived exosome production for clinical applications in regenerative medicine.

Keywords: Extracellular vesicles, Scaling up & manufacturing

PP-490

Investigating Extracellular Vesicle crosstalk using a 3D dynamic model of Breast Cancer

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INTRODUCTION: Extracellular vesicles (EVs) have emerged as promising vehicles for targeted delivery of therapeutic cargo to breast tumours. Biocompatible hydrogels hold immense potential as tunable delivery systems for controlled sustained delivery of EVs to target tissue. 3D in vitro models of cancer are crucial to improve our understanding of this intercellular communication within the tumour microenvironment and beyond to the metastatic niche. The aim of this study was to incorporate EVs into tyramine modified hyaluronic acid (HA-TA) hydrogels for sustained delivery in a dynamic fluidic model system containing breast cancer organoids in alginate gels.

METHODS: HA-TA hydrogels with varying concentrations of hydrogen peroxide and horse radish peroxidase crosslinkers were prepared. Analysis of hydrogel swelling was performed at time points from 5-72 hours. EVs were isolated from plasma using size exclusion chromatography (SEC) and characterized using Nanoparticle Tracking Analysis (NTA) and western blot. Hydrogels were loaded with varying concentrations of EVs, and the rate of release assessed using NTA and protein assay. To create tumour organoids, patient-derived tumour stromal cells were isolated and characterized by flow cytometry. Organoids of GFP labelled MDA-MB-231 breast cancer cells and primary stromal cells were formed in ultra-low attachment plates and loaded in alginate gels cross-linked with calcium chloride. EV uptake by tumour cells was then analyzed.

RESULTS: Analysis of hydrogel swelling revealed a requirement for higher concentrations of HA-TA and crosslinkers to maintain structure and ensure continued swelling. Small EVs in the size range of 30-200nm, expressing EV markers CD63, TSG101 and CD81 were successfully isolated from plasma samples. Plasma EVs were incorporated into the hydrogels with varying release patterns observed over time, depending on EV concentration loaded and hydrogel formulation employed. While using cancer cells in isolation resulted in loose cell aggregates, an admix of a 10:1 ratio of tumour cells: stromal cells resulted in formation of densely packed organoids with budding edges. Over time tumour cells were observed to detach and begin to invade through the alginate gel. Transfer of hydrogel encapsulated/released GFP-EVs to recipient breast cancer cells was observed using confocal microscopy.

DISCUSSION & CONCLUSIONS: Biocompatible hydrogels are employed clinically for multiple applications and hold immense promise in the cancer setting. This initial data supports HA-TA hydrogels as promising systems for incorporation and subsequent release of therapeutic EVs. 3D organoids in alginate gels may mimic the tumour niche. Ongoing research will decipher the crosstalk between the therapeutic EVs and 3D organoids with vasculature in a closed, dynamic fluid flow system.

Keywords: Cancer, Extracellular vesicles

PP-491

Extracellular vesicle microRNA crosstalk between chondrocytes and synoviocytes- an in vitro approach

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INTRODUCTION: Extracellular vesicles (EVs) are crucial in cell-to-cell communication influencing the joint microenvironment. EVs derived from diseased synovial tissue can contribute to osteoarthritis (OA) progression by inducing inflammation and cartilage degradation. EVs contain microRNAs (miRs); powerful regulators of gene expression. To comprehend how they might regulate joint homeostasis, we explored the EV-mediated microRNA exchange in the joint. Our study investigated the EV microRNA crosstalk between equine chondrocytes and synoviocytes in vitro, using RNA tracking and sequencing.

METHODS: Cartilage (n=8) and synovial membrane (n=9) were collected from grossly normal equine metacarpophalangeal joints and chondrocytes and synoviocytes isolated. Cells were incubated with a 5-Ethynyl Uridine tag that labeled newly synthesised RNA. EVs were isolated from culture media using size exclusion chromatography and characterised by nanoparticle tracking analysis. Recipient cells (chondrocytes, n=5; synoviocytes, n=4) were incubated with freshly isolated tagged EVs, ensuring that recipient chondrocytes were incubated with synoviocyte-derived EVs, and recipient synoviocytes were incubated with chondrocyte-derived EVs. Total RNA extracted and 5-EU labelled RNA recovered using an affinity recovery kit, then sequenced.

Processed sequencing reads were mapped using EquCab.3.0 and miRBase v22.1. Differential expression (DE) was analysed using edgeR v3.32 and the quasi-likelihood negative binomial generalized log-linear model function. Results were used for Ingenuity Pathway Analysis.

RESULTS: 198 miRs were identified in synoviocytes, corresponding to the 5-EU labelled RNA in chondrocyte-derived EVs. 26 of these were unique to chondrocyte-derived EVs. A total of 213 miRs were identified in chondrocytes, corresponding to 5-EU labelled RNA in synoviocyte-derived EVs with 41 unique to synoviocyte-derived EVs. The top 5 abundant miRs were similar for both chondrocyte and synoviocyte-derived EVs: miR-21, miR-221, miR-222, miR-100 and miR-26a.

DE analysis revealed a total of 9 miRs, 4 of at FDR<0.05. miR-27b, miR-23b, miR-31, miR-191a and miR-199a-5p, were increased in synoviocyte-derived EVs, while miR-143, miR-21, miR-181a and miR-181b were increased in chondrocyte-derived EVs. A filtered list of 23 experimentally observed targets was obtained, and pathway analysis revealed significant links to migration of cells ($p=3.53 \times 10^{-19}$), angiogenesis ($p=7.79 \times 10^{-17}$) and apoptosis ($p=1.11 \times 10^{-14}$).

DISCUSSION & CONCLUSIONS: There were a higher number of miRs uptaken by the chondrocytes from synoviocyte-derived EVs, as well as a higher number of synoviocyte-specific miRs. This could reflect the increased metabolic activity of synoviocytes. DE miRs appear to be related to cell viability and inflammation reflecting the specific contributions of the joint tissues to these processes.

Keywords: Cartilage / joint and arthritic conditions, Extracellular vesicles

PP-492

Invalidation of *RUNX2* by CRISPR-Cas9 decreases mineralization in three-dimensional cell culture models produced by tissue engineering

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INTRODUCTION: Runt-related transcription factor 2 (*RUNX2*) is a main factor in the regulation of osteoblastic differentiation. It is also linked to the development of calcific aortic valve disease (CAVD). CAVD is characterized by an active remodeling process accompanied with valve mineralization, resulting in a progressive aortic valve hardening and eventual stenosis. The goal of this research project is to study the link between the *RUNX2* gene and CAVD pathogenesis by producing tissue engineering valvular leaflets genetically modified by the CRISPR-Cas9 system. We hypothesize that *RUNX2* knockout (*RUNX2*^{-/-}) in human cells will decrease mineralization in the leaflets following induction of osteoblastic differentiation. The specific objectives are: 1) To produce *RUNX2*^{-/-} cells genetically modified by the CRISPR-Cas9 system; and 2) To study the impact of *RUNX2* invalidation on mineralization, survival and proliferation of different cellular types.

METHODS: The CRISPR-Cas9 double nicking gene editing technique was used to knockout the *RUNX2* gene in different human cell types. *RUNX2* knockout was validated by bioinformatics analysis, Western blotting and indirect immunofluorescence. Proliferation, viability and metabolic activity were also examined. Osteoblastic differentiation was induced and calcification was observed by alizarin red staining and alkaline phosphatase activity. Transcriptomic studies were done using next-generation RNA sequencing approaches.

RESULTS: Different human cell types were successfully edited and knockout for *RUNX2* by the double nicking technique with an efficacy of around 85-100%. *RUNX2*^{-/-} cells have slightly reduced proliferation and viability similar to results found in the literature. After osteoblastic differentiation, mineralization and alkaline phosphatase activity were also significantly decreased. Various other signalling pathways were also investigated in *RUNX2*^{-/-} cells and will be presented.

DISCUSSION & CONCLUSIONS: Invalidation of *RUNX2* by CRISPR-Cas9 decreased mineralization in our three-dimensional cell culture models. The generation of *RUNX2*^{-/-} tissue engineered models will help to better study CAVD pathogenesis, in particular calcification of the tissue. This project will allow us to develop innovative cellular therapy treatments by producing gene-edited and personalized tissue-engineered heart valves on demand using *RUNX2*^{-/-} patient's cells.

Keywords: Disease models, Differentiation

PP-493

A dexamethasone loaded polylactic acid electrospun construct as a tubular cardiovascular implant

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INTRODUCTION: Drug delivery is an important issue for cardiovascular implants. After the implantation procedure the immunological response of the organism is activated provoking a high risk of inflammation and further rejection of the implant from the human organism. Therefore, the existence of an anti-inflammatory drug is needed to assist its constant functionality in the position of implantation. In this study, we produced dexamethasone loaded polylactic acid (PLA) electrospun constructs in a tubular shape for cardiovascular applications.

METHODS: PLA was diluted in a chloroform/trifluoroethanol solving system consisting of 15% PLA and 1- 50 mg/mL dexamethasone. The tubular constructs with 1 mm diameter were produced in a custom-made electrospinning device. The constructs were incubated overnight at 40 °C so that the solvent evaporates and sterilized using UV light for 1 h. For the drug release studies, the constructs were incubated in PBS for 2 months for the calculation of the PLA degradation and the drug release in the solvent through a reference curve. Furthermore, the constructs were used as the protective shields for the molding of tubular human umbilical vein smooth muscle cells (HUVSMCs) and human umbilical vein endothelial cells (HUVECs) embedded fibrin hydrogels which were cultivated for 14 days. The biocompatibility of the constructs was evaluated through a cell proliferation assay. Moreover, the constructs were fixed in 4 % PFA solution and the constructs were stained for α -SMA and CD-31 for the HUVSMCs and HUVECs, respectively.

RESULTS: The percentage of the dexamethasone included in the constructs is proportional to the release in the solvent. The water uptake is proportional of the increasing dexamethasone concentration in the constructs, while the polymer degradation is higher for the higher dexamethasone content of the constructs. The drug release profile created is reflecting an increasing drug release by increasing the dexamethasone concentration. As for the fibrin hydrogel studies, the expression of α -SMA and CD-31 is not affected by the higher dexamethasone concentration. The HUVSMCs and the HUVECs are proliferating and the higher dexamethasone concentration is not limiting their expansion in the construct during the cultivation period.

DISCUSSION & CONCLUSIONS: The combination of the drug-releasing e-spun shield and the effective hydrogel conditioning inside the shield is a dual function system that can be used for cardiovascular applications where a tubular geometry is needed. This system can be useful for embedding of multiple cell types while the drug release can be controlled by using encapsulation methods of dexamethasone.

Keywords: Fibre technology, Polymers - natural / synthetic / responsive

PP-494

Ten-year follow-up study of a mesenchymal stromal cell transplantation clinical trial for stage 3 femoral head osteonecrosis

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INTRODUCTION: Osteonecrosis of the femoral head is a painful disorder that progresses to femoral head collapse and osteoarthritis of the hip joint. The precise pathology remains unknown, but obstruction of blood supply to the femoral head results in necrosis of bone constructing cells. Cell transplantation therapy had been undertaken and good results had been reported^{1,2}. A clinical trial, where cultured autologous bone marrow-derived mesenchymal stromal cells (MSCs) mixed with β -tricalcium phosphate as well as vascularized bone grafts were transplanted to treat stage 3 femoral head osteonecrosis was conducted³. Although an excellent result was obtained, long term results⁴ of cell therapy have rarely been reported. Thus, the aim of this study is to evaluate the long-term effects of MSCs transplantation therapy using radiography, clinical and functional assessments.

METHODS: Participants who underwent MSCs transplantation for stage 3 osteonecrosis of femoral head 10-years ago, were recruited. Femoral head osteonecrosis was radiographically staged using a modified version of the system proposed by the Association Research Circulation Osseous committee. Clinical, hip function, and quality of life scores were estimated.

RESULTS: Radiographic stage was advanced in five hips, two of which had needed total hip arthroplasty. The clinical, function, and quality of life scores were significantly improved and maintained for 10-years.

DISCUSSION & CONCLUSIONS: The effect of MSCs transplantation for advanced stage femoral head osteonecrosis was evaluated 10-years after the treatment. It is useful for maintaining hip function and could be an effective treatment for femoral head osteonecrosis.

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Keywords: Bone and bone disorders (osteoporosis etc), Cell therapy

PP-495

Engineering ureter tissue using hiPSCs

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INTRODUCTION: The ureter is a peristaltic fibromuscular tube that conveys urine from the kidney to the urinary bladder. Congenital anomalies of the ureter (e.g., pelviureteric obstruction, megaureter, and ureteral strictures) can cause hydronephrosis, renal damage and even failure if not treated. In addition, normal ureters can be injured due to trauma, infection and inflammation, renal stones, or due to iatrogenic causes (e.g., post-ureteroscopy stricture or perforation). Treatment options vary depending on the size and site of the affected segment and include ureteroneocystostomy (the ureter is re-implanted in the bladder), ileal ureter replacement (with risk of complications in the urinary and the digestive systems), or ureterostomy (connecting the ureter to a cutaneous stoma, inconvenient especially for young patients). Failure of these techniques would end with undesirable nephrectomy. Engineering ureter tissue could offer a better approach to reconstruct an injured ureter. The advantage of using human induced pluripotent stem cells (hiPSCs) to create ureteric tissue is that they could be generated from any individual and thus they will evade the allogenic rejection that occurs upon transplanting from a donor.

METHODS: We used dissociated mouse ureteric mesenchyme to define growth factors that induce the survival of the ureteric mesenchyme and its differentiation into spontaneously contracting smooth muscles. We then used those growth factors to develop a protocol for inducing the differentiation of hiPSCs into ureteric mesenchyme and functional contractile ureteric smooth muscles. hiPSCs were also differentiated to ureteric epithelium using a sequence of growth factors. Ureteric epithelial tubes were isolated and surrounded by ureteric mesenchymal cells, then were cultured for three weeks to self-organise and produce multi-layered ureter tissue.

RESULTS: hiPSCs were induced into ureteric mesenchyme cells that expressed TBX18. After maturation, the cells showed expression of the smooth muscle markers ASMA, Calponin1, MYH11, and SM22a. Upon prolonged cultures, they developed into spontaneously contracting smooth muscle cells. After co-culturing the ureteric urothelial and ureteric mesenchymal cells, the mesenchymal cells self-organised around the urothelium and developed into few layers of smooth muscle cells. After three weeks of co-culture, a contractile ureter-like tissue developed and showed the expression of UPIII, and KRT5 in the urothelium, and ASMA and Calponin1 in the outer smooth muscle layers.

DISCUSSION & CONCLUSIONS: Our study presents an efficient protocol to differentiate hiPSCs into ureteric mesenchyme and contractile ureteric smooth muscles and shows that correctly differentiated cells can organise themselves forming a multi-layered structure. Self-organisation could be a promising tool for engineering multi-layered contractile ureter tissue.

Keywords: Induced pluripotent stem cells, Kidney

PP-496

Development of Artificial Membrane Binding Proteins for the enhancement of Adoptive Cell Therapies

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INTRODUCTION: Adoptive cell therapies are emerging as an exciting therapeutic modality in medical oncology, due to their novel mechanisms of action that potentially overcome drug resistance and produce durable remissions; these therapies aim to redirect the patient's own immune system to selectively target cancer cells. The lack of widespread treatments for solid tumors, which accounts for 85% of all tumor deaths, stems from the inability of immune cells to effectively home to the tumor site and penetrate the tumor microenvironment. Moreover, a hypoxic and suppressive microenvironment can inhibit cytotoxic cells. The aim of this project is to develop artificial membrane-binding proteins (AMBP) to improve the performance of adoptive cell therapies in solid tumors. AMBPs are proteins that have been modified to spontaneously insert into the membrane of cells, without penetrating into the cytoplasm.

METHODS: In this work, an anchor with oxygen binding capability is rationally designed and supercharged to exert an electrostatically coupling in the membrane. Several biophysical characterization is made to test the oxygen binding functionality of this protein and then, it is fused to a functional domain that exerts an immunomodulatory function. In this regard, an artificial membrane-binding protein consisting of two functional modules is expressed, purified and characterised using several biophysical techniques such as Circular Dichroism, UV-Vis spectroscopy, DLS and MALDI-TOF MS. In addition, oxygen binding assays are performed to demonstrate the functionality of the anchor.

On the other hand, to demonstrate the functionality of the immunomodulator domain, bioluminescence assays are performed and several in vitro functional assays such as cell viability and cytotoxicity will be done.

RESULTS: We were able to obtain the protein in a soluble form with no significant presence of aggregates. Both constructs are correctly folded. The protein of interest is stable up to 45°C, meaning it can be used for therapeutical purposes as it will resist homeostatic human temperature. The bioactivity of the IL15 domain is maintained even after purification and after fusing it to the anchor.

DISCUSSION & CONCLUSIONS: AMBPs can be displayed in the cells and functionalize it with different properties

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Keywords: Cell therapy, Cancer

PP-497

Magnetic Nanoparticles: a novel approach for thermal ablation as treatment of hepatocarcinoma

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INTRODUCTION: Nowadays, cancer has a major impact on societies across the world. In 2020, an estimated 3.4 million new cases of cancer will be diagnosed all over the world. Over 2 million people will die from the disease around the world each year. This work focuses on recent advances in cancer treatment that are overcoming standard techniques, in particular this work focuses on the use of magnetic fields supplemented by magnetic nanoparticles (MNPs). A general procedure for Radiofrequency induction (RF-induction) thermal ablation combined with NPs comprises the following stages: targeted therapy with specific and selective attachment of NPs with cancer, binding validation, and heat administration to the specific area by applying an electromagnetic field [2]. The final target temperatures of hyperthermia is in a range between 42°C (mild hyperthermia) and 90°C (thermal ablation). Today, studies regarding the interaction between magnetic fields generated by radio frequencies and magnetic nanoparticles are focused on scaling up to enhance drug delivery. In this study, we will focus on reaching the temperatures required to reach the cell necrosis.

METHODS: The theory of porous media is used to model the healthy tissue and the cancer tissue. Additionally, to simulate the reduction of blood vessels that flow to the tumor's core, which is a necrotic, a space variable porosity function is implemented. Furthermore, the modulus of velocity vector is assumed to decrease with inverse proportional with the increasing of the damage, reaching a null velocity in the necroted tissue. Different dimensions for nanoparticles ($r = 10\text{--}50\text{ nm}$) are tested. The nanoparticles are exposed to magnetic fields with intensities $H_0 = 5\text{--}23\text{ kA}$ and frequencies $f_0 = 100\text{--}400\text{ kHz}$.

RESULTS: The results show that when the size of nanoparticles increases, the coefficient of diffusivity decreases, and the temperature inside the tumor's dominion rises to reach 48–100°C. Despite an increase in temperature in the healthy tissue domain outside the ablation contour, this does not cause undesired tissue necrosis in the healthy region since the temperature is always above the safety limit. The safety limit is set at 38°C since that is the temperature at which tissue begins to degenerate after prolonged exposure this does not cause undesired tissue necrosis in the healthy region.

DISCUSSION & CONCLUSIONS: This is a brand-new, safe, non-invasive procedure that has the potential to treat cancer.

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Keywords: Advanced therapy medicinal products, Cancer

PP-498

The Effect of Arginine Depletion on Hepatocellular Carcinoma

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INTRODUCTION: Hepatocellular carcinoma (HCC) is the most common primary liver cancer in adults and is the third leading cause of cancer-related deaths worldwide. Common approaches for cancer therapy include chemotherapy and surgery. However, cytotoxicity and non-selectivity of these approaches could damage normal cells. Cancer cells have a high demand for nutrients, like amino acids, to support their rapid growth. Taking advantage of the characteristics of tumors being amino acid auxotrophic, amino acid deprivation therapy has emerged as an effective therapy for many types of tumors. Arginine is a semi-essential amino acid and plays a crucial role in various metabolic processes. It can be depleted by arginase, a mammalian enzyme that converts L-arginine to L-ornithine and urea. Native arginase has a short circulating half-life. We have developed a recombinant human arginase (rhArg) that has an extended circulating half-life. This project aims to investigate the effect of rhArg-induced arginine depletion on HCC and the underlying mechanisms, which may shed light on a new therapeutic strategy for HCC.

METHODS: Two HCC cell lines, HepG2 and Hep3B, were employed as an in vitro model for the study. Cell viability and cell proliferation after treatment with rhArg for various lengths of time was respectively measured by the MTT assay and protein expression levels of proliferating cell nuclear antigen using Western blotting. The effect of rhArg on the cell cycle was analyzed by flow cytometry. Proteomic profiling was conducted using Orbitrap Fusion mass spectrometry. Oxidative stress was determined by measuring the levels of hydrogen peroxide and malondialdehyde, a lipid peroxidation biomarker. Mitochondrial function was analyzed by measuring mitochondrial respiration and energy production using the Seahorse Extracellular Flux Analyzer.

RESULTS: We found that treatment with rhArg markedly reduced cell viability and cell proliferation, and promoted cell cycle arrest at the G2/M phase. Proteomics analysis suggests that mitochondrial function and oxidative phosphorylation pathways are significantly affected by rhArg treatment. Further studies show that rhArg treatment increased the production of reactive oxygen species and enhanced lipid peroxidation. Concomitantly, mitochondrial respiration was greatly suppressed together with a marked reduction in ATP production.

DISCUSSION & CONCLUSIONS: To conclude, rhArg has potent effects on reducing HCC cell viability and proliferation. This could be mediated via induction of cell cycle arrest and an increase in oxidative stress that can impair mitochondrial bioenergetics.

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Keywords: Advanced therapy medicinal products, Cancer

PP-499

Production of radially aligned 3D diaphragmatic constructs through a customized bioreactor

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INTRODUCTION: The production of skeletal muscle constructs useful for replacing large defects in vivo, such as in congenital diaphragmatic hernia (CDH), is still considered a challenge. The standard application of prosthetic material presents major limitations, such as hernia recurrences. With this work, we developed a tissue engineering approach based on decellularized diaphragmatic muscle and human cells for the in vitro generation of diaphragmatic-like tissues as a proof of-concept of a new option for the surgical treatment of large diaphragm defects.

METHODS: Given the myofiber disposition in the diaphragm and the need to force cellular alignment to follow radial spatial orientation, a specific bioreactor system was designed and manufactured in-house to control mechanical stimulation and promote radial stretching during the construct engineering. To perform recellularization experiments, a mixed population of human skeletal muscle cells (hSkMC) and human Fibroblasts (hFb) was injected into decellularized diaphragm ECM.

RESULTS: The effects of dynamic culture were evident from the beginning, influencing transversal cell distribution inside the construct compared with static culture. Enrichment in the epithelial-to-mesenchymal transition and ECM remodeling signaling pathways were found to be overexpressed using GSEA analysis, confirming the positive effect of mechanical stimulation in triggering cell migration. In dynamic samples, the number of injected hFb remained around the physiological ratios, suggesting an active role of mechanical stimulation in controlling hFb overgrowth. Mechanically stimulated constructs also increased tissue maturation, with the formation of newly oriented and aligned muscle fibers. Moreover, after in vivo orthotopic implantation in a surgical CDH mouse model, dynamic constructs maintained the presence of human cells within myofibers and hernia recurrence did not occur, suggesting the value of this approach for treating diaphragm defects.

DISCUSSION & CONCLUSIONS: We generated a diaphragmatic muscle using human cells in vitro and applied mechanical stimulation through a customized bioreactor platform. In vitro tests demonstrated that both ECM remodeling and fibroblast overgrowth were positively influenced by the bioreactor culture. This provided a proof-of-concept of muscle substitute production, laying the foundation for an up-scaled generation of patient specific diaphragmatic constructs for treating large diaphragm defects.

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Keywords: Bioreactors, Decellularised matrices

PP-500

A Bioreactor-based Platform for Investigating the Effects of Direct Perfusion and Pulsed Electromagnetic Field Stimulation on 3D Biomimetic Bone Tissue Models

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INTRODUCTION: Physical stimuli, such as fluid flow-induced shear stress or externally applied pulsed electromagnetic field stimulation (PEMF), strongly influence bone cells proliferation and differentiation and bone mineralization¹. For unravelling genetic pathways activated by specific physical stimuli and involved in bone healing *in vivo* and bone formation *in vitro*, we developed an advanced bioreactor-based platform for investigating the effects of direct perfusion and PEMF stimulation on three-dimensional (3D) biomimetic bone tissue models.

METHODS: The automated bioreactor-based platform², to be incubated, was developed supported by multiphysics simulations (COMSOL Multiphysics), and it is composed of: 3 independent culture chambers (CCs); a controlled perfusion unit (PU); a commercial PEMF stimulator (1.5 mT, 75 Hz). Each 3D-printed CC (Dental SG resin, FormLabs) allows housing a construct of variable size (diameter = 7-10 mm, height = 1-15 mm) and, connected to the PU, exposing it to direct perfusion. The PU consists in a multichannel peristaltic pump, controlled by an Arduino-equipped control box, enabling uni- or bi-directional flow. For biological tests, biomimetic 3D-printed polylactic acid scaffolds, replicating the microarchitecture of trabecular bone³ (average porosity = 65.4% ± 3.3%), were seeded with human mesenchymal stem cells. After 12 h of static culture, the constructs were transferred into the CCs and exposed to uni-directional flow (0.3 ml/min) for 21 days, with or without PEMF stimulation (4 h/day) and using basal (BM) or osteogenic medium (OM) (n=3). Control constructs were cultured statically. The expression of collagen type-I (COL1A1) and type-II (COL2) was assessed by quantitative real-time PCR (qRT-PCR). Transcriptomics analysis is ongoing.

RESULTS: Computational outcomes confirmed that the CC design promotes a symmetric flow profile, avoiding stagnation zones, and the construct is exposed to a uniform magnetic field (1.5 mT).

Direct perfusion and PEMF stimulation, if combined with BM, strongly upregulated the expression of COL1A1 (~6.5-fold change) and COL2 (~4.3-fold change) with respect to static conditions, whereas, when combined with OM, the expression of COL1A1 (~1.2-fold change) and COL2 (~1.7-fold change) was slightly upregulated.

DISCUSSION & CONCLUSIONS: The proposed bioreactor-based platform, combining direct perfusion and PEMF stimulation and allowing parallelization, represents a powerful tool for investigating *in vitro* the biological response of 3D bone tissue models to defined physical stimuli. Biological tests demonstrated the synergic pro-osteogenic effect of direct perfusion and PEMF stimulation,

confirming the value of PEMF stimulation for clinical applications on earth and potentially in space.

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Keywords: Bioreactors, Biomechanics / biophysical stimuli and mechanotransduction

PP-501

**A dynamic culture platform for investigating in vitro the cell response to controlled uniaxial stretch
– Application to periodontal ligament and adipose-derived stem cells**

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INTRODUCTION: Mechanical forces play a crucial role in multicellular organisms, with consequences in cell differentiation, tissue renewal and homeostasis, as well as in pathogenesis¹. In view of mechanotransduction studies, bioreactors designed for replicating *in vitro* defined native-like physical stimuli represent powerful investigation tools². In this study, we developed and characterized a dynamic culture platform for exposing adherent cells to controlled uniaxial stretch, and we tested it with human primary periodontal ligament staminal cells (hPDLSCs) and human adipose-derived stem cells (hASCs).

METHODS: The dynamic culture platform is based on a flexible substrate to be clamped in a previously developed stretch bioreactor³. The design of the substrate (Solidworks), characterized by 2 parallel wells, was optimized by finite element analysis (FEA, Abaqus). Once manufactured by PDMS (Sylgard 184) casting, the substrate was characterized under uniaxial stretch (15% total strain) by digital image correlation analysis (DIC, isi-sys GmbH). For each biological test, 2 substrates were coated with collagen I (Sigma Aldrich) and seeded with hPDLSCs from healthy donors or hASCs (ASC52-telo hTERT, ATCC) (105 cells/well). After 6 hours of static culture, 1 cell-seeded substrate was clamped in the bioreactor and exposed to uniaxial stretch (8% pre-strain + 7% cyclic strain for 90 s, 1 Hz, every 6 h)⁴ for 3 days (n=3). The remaining cell-seeded substrate was cultured statically as control (n=3). Osteogenic markers (ALP, OCN, RUNX2, COL-I) were analyzed by real-time PCR.

RESULTS: FEA demonstrated that, when stretched, the substrate design guarantees an overall uniform strain distribution at the well bottom, as confirmed by the DIC analysis (0.13±0.04 vs 0.15±0.04, respectively). Biological tests showed that hPDLSCs and hASCs respond differently to uniaxial stretch. In comparison to static control, stretched hPDLSCs exhibited an increasing trend of OCN and RUNX2 expression, while for stretched hASCs the markers expression did not significantly change.

DISCUSSION & CONCLUSIONS: The presented dynamic culture platform allows exposing cells to controlled uniaxial stretch while guaranteeing a uniform strain distribution, thus representing a powerful tool for mechanotransduction studies. Biological tests revealed that uniaxial stretch affects hPDLSCs and hASCs differently. Further biological tests imposing different stretch protocols and using pathological hPDLSCs are ongoing, with the final aim to understand the cause-effect relationships between imposed stretch and cell response.

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Keywords: Bioreactors, Biomechanics / biophysical stimuli and mechanotransduction

PP-502

A human multi-organ and dynamic in vitro model for simultaneous and more predictive toxo-efficacy assays

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INTRODUCTION: In the oncology field, the need to address the poor success rate of clinical trials is becoming more and more evident. Therefore, the development of more predictive systems involving dynamic cues and organ-organ interactions is fundamental for evaluating the pharmacodynamic of novel anti-cancer treatments.

A novel multicompartimental and flexible organ-on-chip (OOC) platform has been adopted to fluidically connect a 3D ovarian cancer tissue to a hepatic cellular model, and to resemble the systemic cisplatin administration for investigating drug efficacy and contemporarily measuring potential hepatotoxic effects in a physiological context.

METHODS: Human liver HepG2 and ovarian cancer SKOV-3 cell lines were cultured both in 2D and in 3D, within an alginate based hydrogel [1]. A co-culture model was implemented by cultivating SKOV-3 within porous permeable inserts accommodated within the 24-well plates where HepG2 were plated on the bottom.

A compartmental fluidic device [2] was adopted to assess simultaneously the on-target and off-target cytotoxic activity of cisplatin under dynamic conditions.

For the dynamic multi-organ condition, two independent OOC chambers hosting liver and ovarian cancer models were fluidically connected to each other by the external fluidic circuit, reproducing the in vivo-like tissues arrangement and inter-connections.

RESULTS: A linear decay of both Skov-3 ovarian cancer and Hep-G2 liver cells viability was observed with increasing cisplatin concentration after 48h of treatment. Furthermore, the 3D ovarian cancer model showed a higher drug resistance than the 2D model, when cultured in static condition. Most importantly, the combinatory experimental approach of 3D culture, fluid dynamic condition and multi-organ connection displayed the most predictive toxicity and efficacy outcomes compared to the clinical data.

DISCUSSION & CONCLUSIONS: These results open new challenging opportunities for OOC based approaches in preclinical drug discovery, in the same direction of the 3R based regulatory changing.

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Keywords: Bioreactors, Drug delivery

PP-503

Functionalized Alginate-Hydroxyapatite porous scaffold for Bone Tissue Engineering – Optimization of cell culture conditions

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INTRODUCTION: Bone tissue engineering has emerged as possible alternative to current treatments for bone injuries and defects, but the common 3D static culture approach limits the development of functional bone substitutes, due to insufficient nutrient and metabolite transport and inhomogeneous cell distribution. Flow perfusion bioreactors offer a mechanical stimulation that promotes scaffold cell colonization and osteoblast differentiation in a physical-chemical controlled environment, establishing a favourable condition in view of bone regeneration. To enhance material surface bioactivity, the scaffolds can be then endowed with Fibronectin (FN), an extracellular matrix (ECM) protein involved in the communication between the intra- and the extracellular environment, thus controlling cell behaviour on biomaterials.

METHODS: Cell viability on Alginate/Hydroxyapatite porous scaffolds (Alg/HAp-sc), previously characterized by Porrelli and co-workers, was tested by transposing the MG-63 static culture findings on a dynamic culture and exploring different perfusion bioreactor (IVTech, Italy) set-ups. To overcome the biological inertness of Alginate, scaffolds were afterwards functionalized by soaking into a Fibronectin (FN) solution (10 µg/mL).

Viability studies were performed by Alamar Blue and MTT assays. The insoluble formazan crystals resulted from MTT could be detected by stereomicroscope, while cell morphology was assessed by Environmental Scanning Electron Microscopy (E-SEM). In all cases, 3D printed Poly-(Lactic Acid) (PLA) cages were synthesized to facilitate sample handling during biological assays.

RESULTS: Relevant quantitative and qualitative differences in terms of proliferation rate and scaffold colonization on FN-functionalized scaffolds were observed if compared to Alg-untreated scaffold since the first day in static conditions. These results were confirmed by E-SEM analysis, which showed an optimal cell spreading in the presence of FN with respect to not-functionalized scaffolds, where a round morphology was still prominent. Afterwards, Alg/HAp-FN scaffolds were tested in dynamic conditions, an improved cell viability due to the FN functionalization combined with a more optimized set-up. Indeed, a spindle shape MG-63 morphology was observed also on Alg/HAp-FN scaffolds under dynamic culture.

DISCUSSION & CONCLUSIONS: Overall, these results indicate that the functionalized alginate scaffolds efficiently support cell adhesion and proliferation, showing at the same time adequate structural and physical-chemical properties to be exploited for bone tissue engineering. Further optimization of the culture parameters is still needed as they could influence cell fate and osteogenic differentiation in scaffold-loaded cells cultured in dynamic conditions.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

PP-504

A dynamic in vitro barrier tissue model system for real-time imaging

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INTRODUCTION: The physiological tissue barriers are composed of mainly, epithelial cells and connective tissue which serve to compartmentalise the interacting cells, tissues, and organs. Dysfunction of the barrier leads to pathological consequences. It is important to replicate these barriers when developing in vitro models involving such tissues. Most of the available in vitro models utilise removable inserts fitted in cell culture chambers, which facilitate air-liquid or liquid-liquid interface between the apical and basolateral compartments. Within these compartments, cells or tissues representative of the organ are cultured. However, the current systems do not adequately allow fluid flow on both sides of the barrier, in a physiological manner, and concurrent real-time imaging. We developed the next generation of the Quasi Vivo® chamber system, the new Quasi Vivo® Advance Range, which addresses the major challenges described above, particularly in developing air-liquid (skin, respiratory systems models) and liquid-liquid (intestinal, blood-brain-barrier, blood-retina barrier etc) interface models and allows real-time imaging.

METHODS: A549 cells were seeded on the apical side of a Transwell insert, grown for 4 days before being switched to an air-liquid interface (ALI). Cell counts, viability and barrier integrity were measured. Real-time imaging under flow was also performed.

RESULTS: The cell count, and barrier integrity was increased in dynamic conditions as compared to static conditions and the viability remained the same. We also report the real time microscopy in the form of images.

The key features of the Quasi Vivo® Advance Range system are:

- Portability and easy to use physiological flow system. Allows independent controllable laminar flow of air, gas, or liquid to the apical and basolateral sides.
- Compatibility with real-time microscopy. With optical windows on the top and bottom surfaces of the cell culture chamber for real-time high-resolution imaging.
- Compatibility with most commercially available inserts and models.
- Compact multi-chamber and modular tray format.
- No non-specific binding. This new system is made up of inert and biocompatible resin hence is suitable for pharmacokinetic studies.
- Affordability and easy integration with standard laboratory equipment. Quasi Vivo® Advance Range is inexpensive and is functional inside the standard laboratory incubators and allows real-time fluid sample collection. Access to the biological tissue is possible both during and at the end of experiments.

DISCUSSION & CONCLUSIONS: The Quasi Vivo ADVANCE Range system is an easy-to-use, versatile and adaptable milli-fluidic system and addresses the key challenges faced by the fluidic culture devices: affordability, robustness, and transferability between laboratories.

ACKNOWLEDGEMENTS: Swansea University

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Interfaces - biological

PP-505

Robust Workflows for the Expansion and Differentiation of Human Pluripotent Stem Cells as Aggregates in Suspension Culture

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INTRODUCTION: STEMCELL's TeSR™ 3D-based media products have been developed for robust and scalable suspension culture of human pluripotent stem cells (hPSCs) in aggregates. A critical balance exists between the agitation rate to maintain hPSC aggregates in suspension while minimizing the generation of shear. To date, the field has been challenged by the lack of methods to reproducibly scale hPSCs cultures despite a large range of systems used for less shear sensitive cell types.

METHODS: Six hPSC lines were serially expanded in 9 different suspension culture vessels including 6-well plates on orbital shakers, orbital shaker bottles, and Vertical-Wheel™ PBS-MINI MagDrive Bioreactors. The culture volume varied from 2 mL up to 500 mL. After 3 or 4 days, aggregates were non-enzymatically passaged using Gentle Cell Dissociation Reagent and filter-based trituration. To further validate the suspension workflow, 2 hPSC lines were differentiated into megakaryocytes after 3 passages in suspension cultures. The differentiation protocol used a 12-day endothelial-to-hematopoietic transition phase and a 5-day progenitor-to-mature megakaryocyte stage.

RESULTS: hPSCs underwent ~1.5- to 1.9-fold expansion per day (cell line-dependent) with >85% viability and >90% expression of *OCT4* and *TRA-1-60* in a workflow scaling from 2 mL to 500 mL. The aggregates maintained their capacity to differentiate to three germ layers, while demonstrating uniform morphology and a normal karyotype. The scalable, low-shear Vertical-Wheel™ impeller design yielded reproducible growth across cell lines at the largest growth volumes. Several alternative suspensions, such as spinner and rotating cultures, failed to produce consistent expansion and morphology due to high shear or low mixing. At the end of the erythroblast differentiation protocol, 55 - 90% of cells expressed *CD41a* and 40 - 80% of the cells co-expressed *CD41a* and *CD42b*. Between 15 - 60 *CD41a*⁺ *CD42b*⁺ cells were generated per seeded hPSC (n = 13).

DISCUSSION & CONCLUSIONS: The combination of TeSR™ 3D-based media products and low-shear bioreactors provides a robust system for the expansion of a wide range of hPSC lines. Cells can be readily scaled up from 2-dimensional cultures to 2 mL suspension cultures in 6-well plates, from there up to 60 mL in an orbital shaker bottle, and then into 100 and 500 mL cultures in PBS-MINI MagDrive Bioreactors. These workflows provide a foundation for supplying hPSCs for downstream differentiation with applications to cell therapy, as demonstrated with the megakaryocyte and erythroid protocols.

Keywords: Scaling up & manufacturing, Stem cells - general

PP-506

Biological evaluation of large-scale synthesized superparamagnetic iron oxide nanoparticles

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INTRODUCTION: Superparamagnetic iron oxide nanoparticles (SPIONs) have been widely described for biomedical applications, such as targeted drug delivery, tissue engineering, biomedical imaging or hyperthermia, due to their low toxicity and excellent magnetic properties [1,2]. Among the methods to synthesize SPIONs, flame spray pyrolysis (FSP) technology allows their large-scale production and control of the size and properties [3]. Herein, SPIONs produced by FSP were physico-chemically and magnetically characterized and biological evaluation was performed to explore their potential for biomedical applications.

METHODS: SPIONs were obtained by FSP technology and posterior surface activation treatment and stabilization with dimercaptosuccinic acid (DMSA). In vitro biological evaluation was carried out by (i) cell culture of human bone marrow-derived mesenchymal stem cells (hBM-MSCs) analyzing uptake, viability and ROS content through flow cytometry and confocal microscopy, and gene expression via quantitative real-time polymerase chain reaction (qRT-PCR); (ii) hemocompatibility tests and (iii) microbiological test analyzing the antimicrobial effect against *Escherichia coli* (*E. coli*).

RESULTS: Magnetic SPIONs with 10-18 nm in diameter range have been successfully synthesized by FSP and subsequently coated with DMSA obtaining a time-stable colloidal water dispersion. In vitro assays showed that SPIONs are incorporated by hBM-MSCs in a dose-dependent manner, do not affecting cell viability at the doses studied. No differences with the control were observed in the percentage of intracellular ROS content although it was found a decrease in the intracellular ROS content. Gene expression results showed that SPIONs do not modify Runx2 gene expression and increase ALP gene expression. Hemocompatibility results showed that SPIONs did not produce significant platelet aggregation and suggest a decrease in monocyte activation when SPIONs are coated with DMSA compared to uncoated nanoparticles. Finally, SPIONs were also shown to have a bactericidal effect against *E. coli*, reaching a bacterial inhibition of 95% at a dose of 50 µg/mL.

DISCUSSION & CONCLUSIONS: FSP SPIONs exhibit in vitro biocompatibility and hemocompatibility and their magnetic properties and the analysis of osteogenic Runx2 and ALP genes points to an outstanding behavior for upcoming mechanotransduction applications. Furthermore, the SPIONs possess bactericidal effect against Gram-negative bacteria showing potential for other biomedical applications.

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Keywords: Nanomaterials (inc graphene), Biomechanics / biophysical stimuli and mechanotransduction

PP-507

Vibration Therapy as an Intervention for Enhancing Trochanteric Hip Fracture Healing in Elderly Patients: A Randomized Double-Blinded, Placebo-Controlled Clinical Trial

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INTRODUCTION: Hip fractures are a global concern and poses a huge socioeconomic burden. The treatment of osteoporotic fractures is a major challenge as bone quality is often poor, and pre-clinical studies have shown that healing is delayed. Failure to unite results in pain, weakness, reduced mobility and fixation failure. Therefore, the enhancement of osteoporotic fracture healing has become a major goal in modern fracture management. Low-magnitude high-frequency vibration (LMHFV) is a biophysical intervention that provides non-invasive, systemic mechanical stimulation. Our previous pre-clinical studies have shown that LMHFV accelerates osteoporotic fracture healing. The objective of this study is to investigate the effect of LMHFV on accelerating trochanteric hip fracture healing and functional recovery.

METHODS: A randomized double-blinded placebo-controlled clinical trial was conducted. This study will enrol 120 patients from our institute. Patients aged 65 years or older with an acute trochanteric fracture surgically fixed with a cephalomedullary nail would be recruited, and then randomized into either vibration or placebo group. The LMHFV group would undergo vibration therapy at 35Hz, 0.3g, 20 minutes/day, 5 days/week. Results were evaluated by clinical assessments, radiography with X-rays, Computed Tomography (CT) and dynamic perfusion Magnetic Resonance Imaging (MRI), densitometry, and functional outcomes.

RESULTS: This is an ongoing study. Our preliminary results show that there is an improvement in quadriceps muscle strength. There is also a trend of improved pain control with vibration therapy.

DISCUSSION & CONCLUSIONS: With the ageing population and prevalence of osteoporosis, the enhancement of osteoporotic fracture healing in hip fractures can have significant impact for the recovery of patients. The findings from this randomised controlled clinical study to investigate the use of LMHFV to enhance fracture healing can potentially provide valuable findings for future management with the non-invasive nature of LMHFV.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Translation and commercialisation (inc. clinical trials and regulatory approval)

PP-508

Frontiers in Regenerative Medicine: Compromised Alveolar Bone after Microvascular Reconstructions due to Oncological Diagnosis

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INTRODUCTION: Maxillofacial tumor resections followed by microvascular reconstructions are well established, but often they are associated with a compromised bone architecture regarding volume and structure afterwards. Tissue Engineering and regenerative approaches are extremely challenging in those cases, because the patients underwent for example irradiation procedures. A complete regeneration after surgical oncological therapies is difficult to achieve, but nevertheless, there is an extensive need for dental rehabilitation to restore the masticatory function [1]. It is a relevant question, if ultrashort dental implants might be a successful option to avoid extensive augmentation procedures for that group of patients [2]. The case series evaluated ultrashort dental implants with an innovative macro- and microstructure in compromised bone regarding the implant survival and prosthodontic options.

METHODS: The case series included 14 patients with 61 Bicon implants (Bicon Dental Implants, Boston, USA), which were inserted between 2014 and 2022. Implants were inserted into microvascular anastomosed fibula (6 cases), microvascular anastomosed iliac crest (3 cases), partially resected mandible (3 cases), atrophic maxilla (1 case) and former cleft alveolus (1 case). In 9 of 14 cases the bone underwent an irradiation due to the oncological diagnosis before implant placement. The applied implants measured 5 x 5mm, 4,5 x 6mm und 4 x 6mm (diameter x length) and the average healing time was 6 months. All patients followed a regular recall and the prosthodontic treatment was realized in our department.

RESULTS: All 61 implants were inserted with an acceptable primary stability. The planned and applied prosthodontic treatment was in 13 cases a fixed bridge and in one case a removable denture. 29 months was the average observation period after implant exposure and within this period 13 of 61 implants failed due to loosening. Twelve of the 13 failed implants were inserted into irradiated host bone.

DISCUSSION & CONCLUSIONS: Ultrashort dental implants enabled a stable fixation of dental prosthesis in compromised and atrophic bone without additional augmentation procedures. The risk of implant loss was increased compared to non-compromised, non-irradiated bone. Overall, ultrashort implants represented a useful option for restoration of masticatory, phonetic and aesthetic function in patients with compromised alveolar bone, where the principles of regenerative medicine might getting to a limit.

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Keywords: Cancer, Bone and bone disorders (osteoporosis etc)

PP-509

Haemostatic and genetically augmented wound packing for frontline traumatic soft tissue injuries to prevent bleeding and enhance regeneration

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INTRODUCTION: Haemorrhage is a major cause of mortality in traumatic injury patients. Both military and civilian populations experience haemorrhage as a major cause of mortality following traumatic injury. The patient's survival chances decrease with the amount of blood loss during haemorrhage. Recently, haemostatic materials have changed the way traumatic injuries are treated. Haemostatic materials are the first line of intervention and have the potential to boost survival by stopping bleeding. However, haemostatic materials do not offer any long-term wound healing benefits. A regenerative medicine strategy would be urgently needed to rapidly augment wound repair whilst stopping bleeding. This research fills this need by combining haemostatic materials with regenerative gene therapy that may be used immediately after a traumatic injury.

METHODS: We developed a 3D in vitro model system using a 100 um cell strainer to test the co-administration of gene therapy and haemostatic materials. This involved co delivery the pre-formulated transfection, in liquid format and as freeze dried powder, with the haemostatic material; CELOXTM, directly to the top side of cell strainer with the cells encapsulated with collagen underneath exposed to culture media to ensure cell viability.

RESULTS: This research demonstrates that using GAG- binding enhanced transduction (GET) peptide nanoparticles (NPs) to deliver a Gaussia-luciferase (GLuc) reporter plasmid was successful, both in liquid and freeze-dried formats works in cell monolayers and collagen gels. The haemostatic potential of CELOXTM was maintained in the presence of gene therapy components and was not compromised. This research also demonstrates that the inhibitory action of haemostatic material, CELOXTM, on transfection can be overcome by buffering out the acidity of the CELOX and neutralising the transfection environment making it possible to combine haemostatic material and gene therapy as a regenerative medicine approach in wound repair.

DISCUSSION & CONCLUSIONS: This offers first-responders the option of employing gene therapy and regenerative medicine approaches directly at the point of saving the casualty's life. This will accelerate wound healing and enhance the long-term outcomes following injury.

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Keywords: Wound healing, Gene therapy

PP-510

Optimisation of a Porcine Ex-Vivo Composite Perfusion Model: Applications in Regenerative Medicine

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INTRODUCTION: Ex-vivo composite limb perfusion differs from other perfusion systems as it encompasses multiple tissues. Muscle, nerve, skin, fat, tendon, and vessel functionality can be assessed providing a platform that has numerous applications in the field of regenerative medicine.

METHODS: By performing a series of porcine forelimb experiments, we have developed an ex-vivo living limb system that is capable of restoring circulation for 24hrs (replenishing ATP, preventing cellular injury, and mitigating ischaemia-reperfusion injury) that closely mimics in-vivo physiology. The opportunity to perform paired analyses with contralateral forelimbs creates a realistic platform to assess treatment outcomes with a genetically identical control.

RESULTS: After vessels were flushed with a vasoprotective solution, limbs were perfused for 24hrs via machine perfusion using a blood-based substrate. To isolate key perfusion parameters, we systematically worked to establish a target mean arterial pressure While maintaining physiological blood biochemistry. Following 24 hours of perfusion, histological (skin, muscle, vessel) and molecular analysis indicates that this device provides improved preservation of an isolated limb. We have used thermal imaging as a proxy to successful perfusion and introduced ultrasound imaging to evaluate the health of muscle compartments and to characterise blood flow through the vasculature.

DISCUSSION & CONCLUSIONS: The ex-vivo living limb system has multiple preclinical applications including the development of therapeutic strategies in the management of trauma (burns, blunt trauma and lacerations), controlled fractures, skin drug absorption and wound healing.

Keywords: Trauma / surgery and rehabilitation, In vitro microenvironments

PP-511

The Development of a Porcine Multi-Organ Liver-Kidney-Spleen Model to Advance Research in Tissue Engineering and Regenerative Medicine

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INTRODUCTION: The translation from discovery to tangible therapy that impacts health in a meaningful way remains limited, primarily due to poor laboratory models that have not changed in decades. Microfluidic in-vitro systems offer a poor substitute to in-vivo systems and small animal models rarely offer a route to clinical translation. Large animals offer better translation but are associated with ethical, cost and time restraints. To address this we have developed a multi-organ Liver-Kidney-Spleen system using surplus porcine material, that is capable of maintaining organs in perfect health outside of the body for at least 24 hours.

METHODS: Organs and blood were retrieved from food industry pigs. Relevant vessels were cannulated and organs prepared according to organ donation protocol. Whole blood was cell saved to produce packed red blood cells, which were then mixed with our proprietary perfusion solution. The living organ system was built using clinically approved kit and consumables, and primed with perfusate. Organs were placed in organ chambers, attached to circuit via cannulated vessels and perfused for 24 hours. During this time, haemodynamics and blood biochemistry were continually monitored.

RESULTS: All organs were maintained in optimal health for 24 hours. Haemodynamics, co-oximetry and blood biochemistry remained stable and within physiological parameters. Bile and urine production began immediately and were maintained throughout, indicating restoration of organ function. Macroscopic tissue preservation following 24 hours of perfusion was excellent.

DISCUSSION & CONCLUSIONS: The LIVING-ORGAN system closely recapitulates the in-vivo environment, providing a highly translational, cost effective and ethical alternative to large animal research. It is therapy agnostic, applicable from early proof of concept to complex preclinical modelling. Physiological evaluation of organs can be carried out, producing clinically relevant data such as drug distribution, tropism, uptake, organ specific toxicity, immunogenicity and bioavailability. Future work will consist of further refining the protocol to extend from 24 hours to several days, improving its application as a testing platform. Our aim is to integrate the model within the regular route for drug/therapy development.

Keywords: Bioreactors, Drug delivery



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