TISSUE ENGINEERING PART A
Volume 31, Number 11-12, 2025

https://doi.org/10.1089/ten.tea.2025.90912.abstracts.parte

termis
Tissue Engineering
& Regenerative Medicine
International Society

ABSTRACTS

Abstracts TERMIS EU Freiburg, Germany May 20–23, 2025

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Poster presentation

TERMIS25_1204 Innovative combination of biofermentative unsulfated chondroitin and hyaluronic acid with PRP: assessment of biophysical features and biological effects on osteoarthritis in vitro model. Valentina Vassallo[1], Celeste Di Meo[2], Antonella D'agostino[2], Annalisa La Gatta[2], Donatella Cimini[3], Giuseppe Toro[4], Giovanni Iolascon[4], Maddalena Mastrogiacomo[5], Chiara Schiraldi[2]

[1]Department of Life Sciences, Health and Health Professions, Link Campus University, 00165 Rome, Italy, Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy, Naples, Italy; *[2]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy, Naples, Italy*; *[3]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, 81100, Caserta, Caserta, Italy*; *[4]Department of Medical and Surgical Specialties and Dentistry, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy, Naples, Italy*; *[5]Department of Internal Medicine and Medical Specialties (DIMI), University of Genoa, 16132 Genoa, Italy, Genoa, Italy*

Introduction/Objectives

The most common treatments for Osteoarthritis (OA) are viscosupplementations by using injective intra-articular gels based on glycosaminoglycans (GAGs), particularly hyaluronic acid (HA) and chondroitin sulfate (CS). Recently, also a pharma-grade biofermentative unsulfated chondroitin (BC) was produced by biotechnological process, this latter displayed similar biological effects with respect to CS, into OA in vitro models. This study aimed to assess the viscoelastic features and the relation with in vitro specific biochemical pathways activation (in human chondrocytes isolated from OA joints) of BC combined to high molecular weight HA (HHA/BC) and eventually to platelet rich plasma (PRP). PRP was obtained through a well assessed protocol, characterized at molecular level and lyophilized to achieve stability over time.

Methods

Flow curves and mechanical spectra of HHA/BC alone and HHA/BC+PRP were here obtained by using an oscillatory rheometer. Human chondrocytes were isolated from joints of OA suffering patients replaced by surgical treatment. Primary chondrocytes were cultivated in vitro, for 24 or 48 hours, in presence of HHA/BC (16 mg/mL) diluted 4-fold or HHA/BC (16 mg/mL) + PRP diluted 4-fold and 20-fold respectively. The expression of selected biomarkers related to OA degenerative process was evaluated by quantitative reverse transcription PCR (qRT-PCR), western blotting (WB), immunofluorescence (IF) and multiple ELISA assay. Also, the wound repair ability was valued by time-lapse video-microscopy. Finally, chondrocytes were grown in GAGs-based gels on trans-well for 14 days to mimic a "3D-like" in vitro culture.

Results

HHA/BC+PRP exhibited a consistent rheological profile, providing support for the potential application for intra-articular injections. Furthermore, the maintenance of cell phenotype was confirmed by the analyses of collagen type 2A1 (COLII) and aggrecan (AGN) expression. Still, PRP addition enhanced GAGs ability to reduce specific pro-inflammatory and degradative OA related players (interleukin-6 (IL-6), NF-kB, metalloprotease -13 (MMP-13) and cartilage oligomeric matrix protein (COMP-2)). Both the tested solutions (HHA/BC and HHA/BC+PRP) similarly increased the rate of wound repair.

Conclusions

The outcomes obtained allowed us to better assess the biochemical and biological features of these innovative injectable formulations for potential application in OA management.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1256 Novel formulation based on high and low molecular weight hyaluronic acid and Salvia haenkei for tissue regeneration and pathologies related to the skin. Maria D'agostino[1], Chiara Schiraldi[1], Andrea Maria Giori[2], Antonella D'Agostino[1]

[1]University of Campania "Luigi Vanvitelli", Naples, Italy; *[2]R&D Department, IBSA Farmaceutici Italia, Lodi, Italy*

Objectives: *Salvia haenkei* (SH - Haenkenium®), a native plant of Bolivia, known as strong inhibitor of senescence, also presenting anti-inflammatory properties was recently exploited in wound healing (1,2). Moreover, the use of hyaluronan at high and low molecular weight (HCC), showed to have beneficial effects in dermo aesthetic and regenerative treatments and in pathologies as atopic dermatitis (3,4). Hence, the present research work aims to combine HCC with SH (HCC+SH) to evaluate the ability of this formulation to prompt tissue repair and to prevent skin from dryness. In addition, the anti-inflammatory efficacy of the novel formulation was investigated in an *in vitro* psoriatic model (PS). Specifically, human keratinocytes were challenged with lipopolysaccharides, LPS, derived from *E. coli*, as reported in the recent literature (5). Specific biomarkers and cytokines proved the effect of HCC+SH in reducing the inflammatory mediators/cascade. A more complex model, 3D-full thickness (FT) skin, was used to better resemble an *in vivo* condition, and confirmed the efficacy of novel formulations to counteract the detrimental effects caused by LPS and resembling psoriasis mediators activation *in vitro*.

Methods: Wound healing by time lapse video-microscopy was accomplished to study keratinocytes (HaCaT) migration and thus monolayer scratch repair. Protein expression of tissue remodeling and integrity biomarkers was evaluated by western blotting analyses; dehydration effect was tested by measuring cell viability (cell vitality through MTT test). In *vitro* inflammation status was performed, pretreating HaCaT and 3D FT-skin with novel formulations and after adding LPS at different times. Gene expression by RT-PCR, protein expression by western blotting and immunofluorescence assays were performed for crucial biomarkers in inflammation status, especially related to PS pathology.

Results: Novel formulations prompt wound healing in terms of cell migration and positive modulation of elastin, integrin and aquaporin. Moreover, HCC+SH are effective to counteract the HaCaT from dryness. Inflammatory biomarkers as KRT6, TLR-4 and NF-κB and specific cytokines IL-6, IL-22, IL-23 were significantly reduced by HCC+SH addition. The results obtained using 3D skin were in agreement with the ones obtained with 2D model.

Conclusions: All results trigger the interest in the novel formulation based on SH extract and hyaluronan complexes for its potential efficacy as natural anti-inflammatory agent for damaged skin, for its healing and regenerative properties.

1. Cocetta, V. et al., Aging (Albany NY) 2021, 13(1), 89.

2. Matic, I. et al, Aging (Albany NY) 2016, 8(12), 3223.
3. D'Agostino, A. et al., BMC Cell Biology 2015, 16, 1-15.
4. Zheng, B. W. et al., Life Sci. 2022, 301, 120591.
5. Smolińska, E. et al., PloS one 2018, 13(2), e0192297

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Poster presentation

TERMIS25_1275 Biological response of pharmaceutical grade Triticum vulgare aqueous extract (TVE), in comparison with hyaluronan. Carlo Oliviero[1], Antonella D'agostino[1], Barbara Maglione[2], Chiara Schiraldi[1]

[1]*University of Campania Luigi Vanvitelli, Naples, Italy*; [2]*Farmaceutici Damor S.p.A, Naples, Italy*

Introduction/Objectives

It has been shown that many plant-extracts and/or microbial-derived molecules, with specific relevance of oligo/polysaccharides, prompt tissue repair. The aqueous extract of *Triticum vulgare* (TVE), obtained from a whole germinated plant, with a specific patented extraction fractionation and purification process (1) has been used for many years as an active component in commercial products (Fitostimoline®). TVE is a multicomponent extract with different biological properties, ultimately acting as bioactive complex that directly affects wound repair (2,3). In particular, TVE retentate (containing oligosaccharides with MW>1000 Da) studied in literature for its ability to prompt proliferation of human dermal fibroblast (HDF) and permitted timely wound healing *in vivo* (4). Although there are many scientific reports on TVE functional role, related to clinical studies, a full understanding on the biochemical mechanisms underlying its beneficial effects in tissue regeneration should be further investigated. On this basis, proliferation assay and scratch tests (using time lapse video microscopy station, TLVM) were performed in this study, focusing our attention on re-epithelization biomarkers.

Methods

In a simplified *in vitro* model of wound healing assay, human keratinocyte (HaCaT) or HDF monolayers were scratched, and tissue repair was monitored by TLVM to quantify the healing rate. At the end of the experiment matrix assembly biomarkers (collagen I-A1, collagen III, elastin) were quantified by RT-PCR and proteins expression were performed using western blotting and immunofluorescence assays. In addition, the extract's ability to prompt gene expression of antimicrobial peptides, namely beta defensine (hbd-2) and its biosynthesis (through ELISA assay) has been evaluated. After dehydration (5), survival cells viability was assessed using MTT assay and direct microscopy observation. A comparison was also accomplished with the very well-known hyaluronic acid, using a 200 kDa pharma grade one at concentration resembling the total amount of carbohydrates/oligosaccharides present in TVE.

Results

HaCat and HDF proliferation increased in presence of TVE, similarly to HA addition. TVE increased wound repair of HaCaT cells better than HA added samples. No appreciable difference between the samples was highlighted on HDF monolayer. Concerning HaCaT biomarkers, we found a positive modulation of col I-A1 and HBD-2 gene expressions in presence of TVE treatments, also in comparison with HA. Furthermore, TVE positively stimulated els expression in HDF cell model but this modulation was similar for col I-A1 and col III respect to HA. Elastin, COL I-A1 and COL III protein levels were boosted as observed by immunofluorescence with respect to the CTR. On dehydration assay, TVE increased the capacity of counteract the stressful condition both in HDF and HaCat similar to HA treatments, which is interesting being hyaluronan is a very well-known hydrating agent.

Conclusions

Preliminary results confirmed the beneficial biological properties of TVE, resembling those of HA, also in terms of activated biochemical pathways and key biomarkers. Besides, an interesting outcome for defensin upregulation never reported to date was found.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1279 The Tumor Microenvironment: A Crucial Player in Head and Neck Cancer Development. Emilia Reszczyńska[1], Arkadiusz Czerwonka[1], Aanchal Chopra[1], Adrian Andrzejczak[1], Alinda Anameric[1], Tomasz Stankiewicz[2], Matthias Nees[1]

[1]Medical University of Lublin, Lublin, Poland; *[2]Saint Jan of Dukla Oncology Centre of the Lublin Region, Lublin, Poland*

Introduction/Objectives

Head and neck squamous cell carcinoma (HNSCC) arise from the stratified epithelium of the upper gastrointestinal tract and constitute as much as 90% of all head and neck cancers. Their development is most often promoted by risk factors such as smoking, papillomavirus (HPV) infection, excessive alcohol consumption or genetic predisposition. Therapy options of HNSCC have not improved for decades, and typically consist of radiotherapy for primary tumors, followed by more aggressive chemotherapy for recurrent, metastatic cancers. State of the art chemotherapies typically comprise cisplatin (CDDP), 5-fluorouracil (5-FU), and Cetuximab (a recombinant antibody targeting EGF receptor). In addition, it is now known that stromal properties and the tumor microenvironment (TME) such as cancer-associated fibroblasts (CAFs), tumour-stroma interactions, and the tumor immune microenvironment (TIME), define the success or failure of these therapies. In addition, the epithelial-mesenchymal transition (EMT) and the presence of cells that undergo a partial EMT (pEMT) plays an important role in cancer progression, distant metastasis, and sensitivity versus acquired resistance to chemo-radio-therapies.

Methods

Our study used complex cell suspensions isolated from fresh tumors, mainly from the tongue and oral cavity. Isolation of cell suspensions from biopsies was based on collagenase IV, followed by differential two- and three-dimensional (2D and 3D) cell culture techniques to isolate CAFs from different types of tumour cells (pEMT and squamous carcinoma cells), using chemically defined preparations of extracellular matrix (ECM), and media with different composition, serum content, and growth factors. This allowed us to systematically co-culture tumor and stromal cells in a persistent, controlled fashion. Both 2D without matrix, and 3D cultures of tumor cells suspensions on top of defined ECM preparations differentiate spontaneously into complex microtissues that resemble the histology and architecture of HNSCC. These 2D and 3D microtissues mimic the histology and the TME of the original tumor biopsies better than HNSCC cell lines. Specifically, tumor-like microtissues, or microtumors, generated in both 2D and 3D conditions, were then used to test the sensitivity of chemotherapeutic agents CDDP and 5-FU. Expression of epithelial versus mesenchymal and endothelial biomarkers were carried out using immunofluorescence (IF) antibody staining.

Results

Our results showed that CDDP and 5-FU, applied to patient-derived microtissues cultured in both 2D and 3D conditions, showed differential drug sensitivity. This was independently confirmed by cell proliferation assays. In addition, post-drug labelling of cells in our 2D and 3D microtissues showed gross changes in cell morphology after chemotherapeutic treatment. In addition to cell proliferation, significant and characteristic drug-induced changes in protein expression levels (e.g. vimentin, α-smoth muscle actin, cytokeratin-14) were observed by IF and Western blotting assay.

Conclusions

Both the 2D and 3D models showed that the efficacy of the drugs were similar, but IC50 was lower for microtissues on top or embedded in 3D ECM, compared to 2D monolayer co-cultures. Furthermore, we show that the same therapy can produce highly differential results in different patients, and multiple factors define drug sensitivity versus resistance.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1282 Cell-assembled matrix sheets produced with 2-phospho-L ascorbic acid.

David Brownell[1], Laurence Carignan[2], Reza Alavi[2], Christophe Caneparo[1], Maxime Labroy[1], Stéphane Chabaud[1], Todd Galbraith[1], François Berthod[1], Laure Gibot[3], François Bordeleau[1 2], Stéphane Bolduc[1 4]

[1]Laboratoire d'organogénèse expérimentale (LOEX), CRCHU de Québec, Université Laval, Québec, Canada; *[2]Centre de recherche en cancer (CRC), CRCHU de Québec, Université Laval, Québec, Canada*; *[3]IMRCP, CNRS, Université Paul Sabatier, Toulouse, France*; *[4]Département de chirurgie, Faculté de médecine, Université Laval, Québec, Canada*

Introduction/Objectives

Ascorbic acid is a critical building block of collagen. Its deficiency is associated with impaired extracellular matrix deposition. Thus, sodium ascorbate (AA) is often added to culture media in tissue engineering applications to stimulate the production and maturation of an endogenous extracellular matrix by the fibroblasts. Production of various tissues by the self-assembly technique relies on the ability of stromal cells to produce their extracellular matrix. The use of AA is therefore crucial. However, AA is unstable in aqueous solution, even when stored at 4°C, meaning that fresh solutions must be made at every medium change. This poses a challenge to produce engineered tissues as it increases cost and manipulation time. A more stable form of ascorbate, 2-Phospho-L-Ascorbate (2PAA), allows the formation of connective tissue-like structures in fibroblast cultures. It remains unclear if 2PAA constitutes an adequate substitute to sodium ascorbate. In this context, we evaluated the physico-chemical properties of AA or 2PAA solutions and how 2PAA impact the properties of dermis, bladder and vaginal tissues reconstructed using the self-assembly technique.

Methods

Sodium ascorbate (AA) and 2-phospho-L-ascorbate (2PAA) stability was assessed for a working dilution (50µg/ml) under storage conditions (4°C or -80°C) as well as under culture conditions (37°C humidified 5% CO2 incubator). Solution samples were taken at different time points and measurements were performed on a UV–vis scanning spectrophotometer. Engineered dermis, bladder and vaginal tissues were produced using primary tissue-specific fibroblasts. Cells were seeded in 6-well plates with a paper anchor and weight. Cells were cultured with 50µg/ml AA or 2PAA. After 4 weeks, 3 stromal sheets were stacked and fused for 4 days. Mechanical characterization was performed on an Instron Electropuls®E1000. Stroma organization was assessed with histological staining. Enzymatic activity of matrix metalloproteinases (MMPs) was assessed. Finally, a price analysis was performed.

Results

Direct UV-Vis spectrophotometry showed a maximum of absorption around 260 nm for both freshly prepared AA and 2PAA solutions. After 15 to 30 days at 4°C, the AA solution underwent yellowing along with a shift of the maximum absorption peak, indicating degradation. To a lower extent, this was also observed for the AA solution at -80°C. No degradation was observed for the 2PAA solution. Over 72h at 37°C (culture conditions), even faster change in the color and spectrum of AA was observed while 2PAA remained stable.

We then tested if tissues produced using 2PAA were equivalent to those obtained with AA. Notably, tissues produced with 2PAA had comparable thicknesses and equivalent mechanical properties compared to those produced with AA. MMP activity revealed no significant difference between AA and 2PAA, and collagen content and organization were found to be similar. A price comparison also revealed that 2PAA ($0.51 /$cm^2$) might be particularly advantageous compared to freshly made AA ($4.79/$cm^2$).

Conclusions

Overall, our results demonstrate the possibility of substituting sodium ascorbate with 2-Phospho-L-Ascorbate for tissue engineering using the self-assembly approach. This protocol would significantly reduce the costs associated with tissue production while lowering required hands-on time. These are key factors for any scaling activity.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1293 Multi-component system for enhanced osteochondral tissue engineering.

Odeta Baniukaitiene[1], Aiste Pupiute[1], Edvinas Krugly[1], Darius Ciuzas[1], Jokubas Lukosiunas[1], Dainius Martuzevicius[1], Eiva Bernotiene[2], Edvardas Bagdonas[2], Tomas Ragauskas[2], Vaiga Žemaitienė[2]

[1]Kaunas University of Technology, Faculty of Chemical Technology, Kaunas, Lithuania; *[2]State Research Institute Center for Innovative Medicine, Vilnius, Lithuania*

Introduction/Objectives

Osteochondral tissue engineering offers a promising approach for repairing cartilage and subchondral bone injuries, which are challenging to heal due to the complex structure and limited regenerative capacity of osteochondral tissue. The main objective of this work is to develop a multi-component system for osteochondral tissue engineering, combining fibrous gradient-pore scaffold integrated with hyaluronic acid-based hydrogel. This multi-component system aims to mimic the native osteochondral environment, providing structural support while guiding cellular growth and promoting tissue regeneration.

Methods

Fibrous gradient-pore scaffolds of polycaprolactone (PCL) were produced using the Bious Labs 3D fiber printer (3Df-01C, Bious Labs, Lithuania), which integrates melt electrospinning and fused deposition modelling technologies. Surface modification of the scaffolds was performed using non-thermal plasma treatment to enhance hydrophilicity (DBD-01-V, Bious Labs, Lithuania). Bioactive hydrogels of modified hyaluronic acid, incorporated with cell-stimulating growth factors, were immobilized into the fibrous scaffolds. The release kinetics of growth factors were studied using enzyme linked immunosorbent assay (ELISA). Cell proliferation within the multi-component system was assessed using the CCK-8 (Cell Counting Kit-8) assay.

Results

Fibrous gradient-pore scaffolds of PCL were obtained using a combination of melt electrospinning and fused deposition modeling. It was determined that non-thermal plasma treatment can enhance the hydrophilicity of the scaffolds, providing suitable conditions for hydrogel infusion. Additionally, it was found that cell-stimulating growth factors are gradually released from the hydrogel, demonstrating a significant effect on cell proliferation within the multi-component system.

Conclusions

The bioactive hydrogel, immobilized within the polymeric scaffold, gradually released cell-stimulating growth factors, significantly enhancing cell proliferation. These findings indicate that the developed multi-component system offers strong potential for tissue regeneration by providing both structural support and bioactivity.

Acknowledgements

This study was partially supported by the Research Council of Lithuania under Grant P-MIP-24-498.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1296 Micropatterned styrene-butadiene-styrene thin films doped with barium titanate nanoparticles: effects on myoblast differentiation.

Leonardo Boccoli[1], Elena Drago[1], Angelo Sciullo[1], Andrea Cafarelli[1], Federica Iberite[1], Lorenzo Vannozzi[1], Sajedeh Kerdegari[2], Toshinori Fujie[3], Emanuele Gruppioni[4], Claudio Canale[2], Leonardo Ricotti[1]

[1]Scuola Superiore Sant'Anna, Pontedera, Pisa, Italy; *[2]Università di Genova, Genova, Italy*; *[3]Tokyo Institute of Technology, Tokyo, Japan*; *[4]Centro Protesi INAIL, Bologna, Italy*

Introduction/Objectives

Polymeric thin films featured by a submicrometric thickness are gaining interest in tissue engineering due to their high surface-to-thickness ratio, flexibility, and tunable mechanical and topographical properties[1]. They can be easily patterned with anisotropic cues that guide the alignment and differentiation of skeletal muscle precursor cells, favoring muscle tissue development. Piezoelectric nanomaterials are also promising, especially for skeletal muscle tissue engineering, as electric stimuli generated by activated piezoelectricity may facilitate muscle differentiation and contraction[2]. This study evaluates the potential of barium titanate nanoparticle (BTNP)-doped poly(styrene-butadiene-styrene) (SBS) thin films as engineered anisotropic supports for myogenic differentiation.

Methods

BTNPs (average diameter: 300 nm) were embedded at a concentration of 1% w/v in 40 mg/ml SBS thin films fabricated via spin-coating on micropatterned PDMS masks with evenly spaced channels of 10 μm width and 1 μm depth. Atomic force microscopy was used to assess mechanical properties through nanoindentation, while immunofluorescence imaging and gene expression analyses were conducted to evaluate the myogenic differentiation of C2C12 cells.

Results

The thin films exhibited submicrometric thickness (≈ 0.8 μm) with a flexural rigidity of 3.43×10^{-12} N·m, and regular anisotropic grooves, which promoted cell alignment and myotube formation. Notably, embedded BTNPs enhanced the expression of myogenic markers (e.g., MYH1, MYH4, MYH8, ACTA1) after 10 days of culture, supporting late-stage myogenic differentiation of C2C12 cells, but without significantly impacting mechanical properties.

Conclusions

BTNP-doped SBS thin films proved effective as flexible, anisotropic substrates supporting myogenic differentiation, by exploiting piezoelectricity. This approach may facilitate non-invasive, ultrasound-mediated electrical stimulation of muscle cells for muscle tissue engineering, or biohybrid actuators. Moreover, these substrates represent a versatile quasi-2D platform suitable for both muscle monocultures and co-culture systems (e.g., neuromuscular ones).

Acknowledgment: This work received financial and technical support from INAIL, project MioPRO2, grant n.PR23-CR-P1.

[1] Fujie, T. Development of Free-Standing Polymer Nanosheets for Advanced Medical and Health-Care Applications. Polymer Journal 2016, 48 (7), 773–780. https://doi.org/10.1038/pj.2016.38.

[2] Paci, C.; Iberite, F.; Arrico, L.; Vannozzi, L.; Parlanti, P.; Gemmi, M.; Ricotti, L. Piezoelectric Nanocomposite Bioink and Ultrasound Stimulation Modulate Early Skeletal Myogenesis. Biomater. Sci. 2022, 10 (18), 5265–5283. https://doi.org/10.1039/D1BM01853A.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1306 Biofabrication of Cardiac Constructs: Evaluating PHA Scaffolds for Myocardial Infarction Therapy. José Valdés Fernández[1], Iker Arrizabalaga Asorey[2], Olalla Iglesias-García[1 3], Eduardo Larequi[3 4], Ilazki Anaut-Lusar[1 3], Oihane Delgado Zabala[5], Vicente López[5], Felipe Prósper[3 6 7], Mariola Calle de Celis[2], Manuel M. Mazo Vega[1 3 4]

[1]Biomedical Engineering Program, Enabling Technologies Division, CIMA Universidad de Navarra, Foundation for Applied Medical Research, Pamplona, Spain; *[2]Surface Technology and Advanced Materials, Asociación de la Industria Navarra, Cordovilla, Spain*; *[3]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain*; *[4]Hematology and Cell Therapy, Clínica Universidad de Navarra, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain*; *[5]Centro Nacional de Energías Renovables (CENER), Sarriguren, Spain*; *[6]Hemato-Oncology Program, Cancer Division, CIMA Universidad de Navarra, Foundation for Applied Medical Research, Pamplona, Spain*; *[7]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC) CB16/12/00489, Madrid, Spain*

Introduction/Objectives

Cardiovascular diseases (CVDs) remain the primary cause of global morbidity and mortality. Among them, myocardial infarction (MI) is marked by an abrupt blood flow interruption to a region of the heart, causing oxygen and nutrient deprivation and subsequent tissue death. This event triggers a gradual, progressive decline in cardiac function. The lack of treatments to restore heart function emphasizes the need for lab-generated human cardiac tissue. This study evaluates P-3HB-co-4HB (PHA) as a material for biofabricating engineered human myocardium with therapeutic potential. The key objectives are:

1. Fabricate PHA scaffolds via Fused Deposition Modelling (FDM) 3D printing and solvent casting techniques.

2. Develop 3D cardiac constructs using PHA scaffolds, comparing their performance with medical-grade poly-ε-caprolactone (PCL) as a gold standard.

3. Optimize PHA scaffold structures to enhance human induced pluripotent stem cell-cardiomyocyte (hiPSC-CM) attachment, organization, maturation, and functionality.

4. Evaluate the therapeutic efficacy of PHA constructs in a rat MI model.

Methods

Rhomboidal PCL/PHA scaffolds were fabricated using FDM 3D printing, while porous film PCL/PHA scaffolds were created through solvent casting and perforation. Scaffold biocompatibility was assessed with Alamar Blue and Live/Dead assays. To form 3D cardiac constructs, two million hiPSC-CMs were embedded in a fibrin hydrogel and cast onto rhomboidal or porous film PCL/PHA scaffolds. Construct viability was tested at days 1, 7, and 14 using the Alamar Blue assay. Structural and functional analyses included measuring beating rates, qPCR for cardiac markers, and immunofluorescence for structural proteins. Therapeutic potential was tested in an athymic rat MI model.

Results

Rhomboidal PHA scaffolds were successfully fabricated via FDM 3D printing. Biocompatibility tests showed PHA scaffolds supported cell survival at levels comparable to PCL scaffolds. FDM-printed rhomboidal scaffolds formed stable cardiac constructs, maintaining hiPSC-CM viability for 14 days. However, their large fiber diameter caused excessive construct rigidity, limiting hiPSC-CM contractility, with contraction observed ocassionally in the hydrogel. qPCR indicated overall downregulation of cardiac markers, reflecting suboptimal scaffold performance. Adjusting the structure to solvent-cast flat films improved contractility but reduced cell adhesion, leading to detachment and declining viability and

functionality. Adding pores to the films significantly enhanced cell attachment, yielding contractile scaffolds that maintained viability and functionality. These scaffolds improved cardiac function in a rat MI model, reducing early inflammation and subsequent fibrosis, demonstrating the therapeutic promise of this approach. Further studies are required to evaluate the impact of cardiac PHA constructs as a therapeutic candidate for MI.

Conclusions

PHA scaffolds show excellent biocompatibility. While FDM printing produces overly rigid constructs unsuitable for mimicking myocardial contractility, porous film scaffolds generate flexible, contractile constructs that sustain hiPSC-CM viability. In vivo tests will further evaluate the suitability of porous PHA scaffolds for MI treatment.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1319 Design and Evaluation of a Miniaturized Bioreactor for Osteochondral Tissue Engineering. Francesco Carfì Pavia[1], Camilla Carbone[1], Gundula Schulze-Tanzil[2], Clemens Gögele[2], Elisa Capuana[1], Valerio Brucato[1], Vincenzo La Carrubba[1]

[1]*University of Palermo, Palermo, Italy*; [2]*Paracelsus Medical University, Nuremberg, Germany*

Introduction/Objectives

Osteochondral tissues, comprising articular cartilage and subchondral bone, pose a significant regenerative challenge in regenerative medicine. Traditional approaches often fail to restore complete functionality, underscoring the importance of tissue engineering (TE) solutions combining scaffolds, cells, and dynamic culture systems. This study focuses on the scale-down and optimization of a previously developed external-loop airlift bioreactor prototype, for dynamic cell culture aiming to enhance human mesenchymal stromal cells (hMSCs) growth on bilayer scaffolds specifically designed for osteochondral tissue repair.

Methods

The bioreactor was printed using stereolithographic 3D printing, allowing modular and flexible design workflows in Autodesk's FUSION 360 software. Compared to the original glass prototype, the miniaturized version achieved a 70% reduction in height and an 80% reduction in perfusion chamber diameter, resulting in final dimensions of 6.5 cm height, 1.8 cm diameter. These modifications substantially lowered the required medium volume from 60 ml in the original prototype to 8 ml, while maintaining the capacity to host the same number of samples. Computational Fluid Dynamics (CFD) simulations were carried out to optimize the fluid dynamics of the system, with the aim to obtain an homogeneous flow in the whole bioreactor and at the same time to avoid cell detachment. Finally, a global oxygen mass balance model was developed to evaluate the oxygen transport, a crucial factor for cell culture, inside the bioreactor.

To validate bioreactor performance, poly-L-lactic acid (PLLA) bilayer scaffolds produced via Thermally Induced Phase Separation (TIPS) technique were utilized. The scaffolds, with distinct pore sizes (100 μm in the cartilage-mimetic layer and 250 μm in the bone-mimetic layer), were filled with hydroxyapatite (HA) to improve osteoconductive properties. Murine fibroblasts (L929) and hMSCs cells were seeded under static and dynamic conditions. Seeding efficiency, cell viability, cell morphology and migration inside the scaffolds were monitored via Cell-titer Blue, Live/Dead assay Histological staining. Moreover, the presence of type I and II collagen was investigated via Immunostaining.

Results

The fluid flow rate was optimized through CFD simulation, ensuring system-wide homogeneity and consequently a uniform nutrient distribution while minimizing turbulences, potentially critical for cell

culture. Transient and steady-state oxygen concentration profiles validated the bioreactor's capacity to support oxygen transfer from gas to liquid phases. With an air inlet velocity of 8,2*10-2 m/s, a 8 mL culture medium volume, and a cell density of 360,000 cells, the system maintained sufficient oxygen levels to support metabolic activity. Dynamic culture significantly improved cell viability, metabolic activity, and extracellular matrix production compared to static culture. PLLA+HA scaffolds demonstrated higher performance over pure PLLA scaffolds in terms of cell attachment and proliferation.

Conclusions

The presented bioreactor offers a cost-effective, scalable platform for high-throughput testing of cell-scaffold constructs, aligning with emerging trends in TE miniaturization and automation. Future studies will employ this system for co-culture scenarios with chondrocytes and mesenchymal stromal cells, advancing its potential in osteochondral repair applications.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1403 Biofabrication of a stromal-epithelial substitute of the human urethral mucosa by tissue engineering. Jesús Chato-Astrain[1], Miguel Etayo-Escanilla[1], Paula Ávila-Fernández[1], Ingrid Garzón[1], Esther Liceras-Liceras[2], Eloísa Díaz-Moreno[2], Miguel Alaminos[1], M. Carmen Sánchez-Quevedo[1], Ricardo Fernández-Valadés[3], David Sánchez-Porras[1]

[1]University of Granada, Spain, Instituto de Investigación Biosanitaria ibs.GRANADA, Spain, Granada, Spain; *[2]Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain, Granada, Spain*; *[3]Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain, Instituto de Investigación Biosanitaria ibs.GRANADA, Spain, Granada, Spain*

Introduction/Objectives

The human urinary mucosa is a biological structure in direct contact with the urine expelled through the urethra, and its alteration is typically associated with the generation of a stricture that is very difficult to manage. Although some substitutes of this structure have been described, fully efficient artificial substitutes allowing optimal *in vivo* regeneration of the urethral mucosa have been seldomly described [1]. The epithelial layer of the urethra is a complex specialized tissue, which increases the difficulty of *in vivo* regeneration and *in vitro* recreation. In this sense, the present study raises the possibility of generating *in vitro* urethral mucosa substitutes using fibrin-agarose hydrogels, a biomaterial that has shown promising results to support growth of epithelial cells of different origin, such as the ureter [2].

Methods

Mucosal substitutes were generated by a three-step biofabrication process. First, mucosal stroma was fabricated by encapsulating fibroblasts in a fibrin-agarose hydrogel. Once jellified, epithelial cells were seeded on the surface of the stroma and allowed to grow for 2 days using immersed culture conditions. To induce epithelial stratification, the monolayer epithelium was exposed to intermittent air-liquid conditions to resemble native conditions for 12 days. Viability, proliferation, and expression of cytokeratins and cell-junction proteins were assessed.

Results

Histology revealed the presence of viable proliferative cells. Epithelial cells showed potential to grow and differentiate on the fibrin-agarose hydrogels, increasing their number with the culture time. Furthermore, it was also observable a maturation process, showing several differentiation features, with a basal stratum (positive to cytokeratin 14), an intermediate layer (positive to cytokeratin 7), and apical superficial layer of cells with the characteristic umbrella morphology. Moreover, these cells showed positive reaction to epithelial cell adhesion protein desmoplakin.

Conclusions

Our results reveal that fibrin-agarose is a promising scaffold for generating artificial urethral mucosa substitutes with high levels of biomimicry to the native urethra. However, more research is still needed to determine its potential for the treatment of urethral pathologies.

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, Instituto de Salud Carlos III (grant FIS PI22/00059). Co-financed by the European Regional Development Fund (ERDF) through the "Una manera de hacer Europa" program, European Union.

References:

[1] Elia, E., *et al* (2024). Tissue Engineering for Penile Reconstruction. Bioengineering, 11(3):230.

[2] Jaimes-Parra, B. D., *et al*. (2016). Ex vivo construction of a novel model of bioengineered bladder mucosa: A preliminary study. Int. J. Urol., *23*(1), 85-92.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1468 Phenotypic characterization of the NANOULCOR human bioartificial cornea model based on fibrin-agarose biomaterials. Jesús Chato-Astrain[1], Olimpia Ortiz-Arrabal[1], Miguel Etayo-Escanilla[1], David Sánchez-Porras[1], Óscar Darío García-García[1], Carmen González-Gallardo[2], Miguel Ángel Martín-Piedra[1], Ingrid Garzón[1], Miguel Alaminos[1]

[1]*University of Granada, Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*; [2]*Division of Ophthalmology, University Hospital San Cecilio, Granada, Spain*

Introduction/Objectives: Different models of human bioartificial cornea have been generated by tissue engineering, although very few of them reached the clinical setting and were evaluated in patients. One of the cornea substitutes showing clinical potential is NANOULCOR, generated with human cornea epithelial and stromal cells (keratocytes) immersed in nanostructured fibrin-agarose hydrogels. Although this corneal substitute demonstrated to be useful in patients with central cornea damage, its potential usefulness in patients with associated damage of the scleral limbus has not been demonstrated. The objective of this work is to characterize the histological, molecular and genetic pathways that are activated in these cornea substitutes in order to determine the potential clinical applications of this advanced therapies medicinal product (ATMP).

Methods: To generate the NANOULCOR substitutes in the laboratory, we first fabricated a stromal substitute with human cornea keratocytes cultured within fibrin-agarose biomaterials. Then, a corneal epithelium was generated by subculturing human cornea epithelial cells isolated from human scleral limbus biopsies on top of the stromal substitute [1]. These tissues were kept in culture for 28 days, using air-liquid culture techniques from day 21 to favor epithelial differentiation. NANOULCOR samples were histologically analyzed to determine the structure of the corneal epithelium and its degree of biomimetism as compared to native human corneas. Relevant histological, molecular and genetic pathways related to corneal differentiation were analyzed by immunohistochemistry for pancytokeratin (PCK), cytokeratin 5 (KRT5), crystallin lambda (CRYλ) and the cell-cell junction protein TJP1, and the markers of undifferentiated limbal stem cells keratin 3 (KRT3) and the limbal isoform of the protein p63 (ΔNp63). Human central cornea and limbus were used as control, and the expression found in these tissues was compared with that of NANOULCOR.

Results: Results confirmed that NANOULCOR had a stratified epithelium on top of a stromal substitute consisting of keratocytes immersed in a dense fibrillar biomaterial, and displayed histological similarities with the native cornea. For the expression of epithelial cell markers, all samples were positive for PCK,

KRT5, CRYλ and TJP1, although the expression levels were lower in NANOULCOR than in central cornea control tissues for KRT5 and CRYλ. For the limbal stem cell markers, we found that NANOULCOR showed similar expression levels than control limbus for KRT3 and ΔNp63, suggesting that this epithelium was partially biomimetic to that of the human limbus.

Conclusions: These results suggest that NANOULCOR epithelium shares important structural, molecular and gene expression similarities to the corneal limbus, and could retain some of the regenerative potential of this structure. These findings suggest that NANOULCOR could be potentially used in patients with associated limbal deficiency.

This work was supported by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities, grants FIS PI23/00335 and ICI21/00010 (NANOULCOR). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

[1] Rico-Sánchez, L., et al. (2019). Successful development and clinical translation of a novel anterior lamellar artificial cornea. *J Tissue Eng Regen Med* 13, 2142–2154

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1475 Recreation of 3D Skeletal Muscle Conditions Using Graphene Microelectrode Arrays.

Maria Crespo-Cuadrado[1], Miquel Madrid-Gimeno[2], Florencia Lezcano[1], Maria Martí Carrascosa[2], Elena del Corro[2], José Antonio Garrido[2 3], Samuel Sánchez[1 3]

[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain; *[2]Catalan Institute of Nanoscience and Nanotechnology (ICN2), Barcelona, Spain*; *[3]Institució Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Spain*

Introduction/Objectives

Skeletal muscle tissue plays a crucial role in body posture and movement, impairing people’s quality of life after muscular damage. Tissue-engineered skeletal muscle models have opened the opportunity to understand the biological pathways associated to specific muscle conditions. However, recreation of local muscle ruptures occurring during muscular overextension is still limited. Similarly, local rehabilitation of severe muscle injuries is hindered by the unspecificity of conventional treatments. To overcome these challenges, we present the incorporation of graphene microelectrode arrays as new tool for the modelling and treatment of clinically relevant muscle conditions.

Methods

C2C12 mouse myoblasts were encapsulated in a hydrogel comprised of 30% Matrigel and 4mg/mL fibrin. Cells were casted in 3D printed circular molds and cultured for three days, before transferring them to a two-pillar system. Muscular differentiation was achieved after culturing the tissue constructs in differentiation media. As benchmark of graphene microelectrodes effect on muscle models, their effect on muscle tissue was compared with standard bulk graphite electrodes. A biphasic pulse with a frequency of 1Hz was selected for the recreation of twitch contractions, whereas monophasic pulses with 50Hz frequency was introduced to mimic muscle tetanic contractions. The effect of electrical stimulation on myotubes through the model was assessed by immunostaining of actin filaments and cell nuclei. Finally, the effect of muscle stimulation on gene expression was assessed by Real-Time Polymerase Chain Reaction of myogenic transcription factors.

Results

The use of conventional graphite electrodes resulted in the contraction of the whole muscle construct. Conversely, the incorporation of graphene microelectrode resulted in the muscular contraction of only those tissue regions closer to the graphene microelectrodes. Despite the muscle response was localized only in

these specific regions, the observed contractions followed the frequencies defined in the stimulation protocols. The close contact between the microelectrode arrays and the muscular fibres could explain the local stimulation in these tissue areas, in contrast to the unspecific stimulation with standard electrodes, which requires the transmission of electrical current through the media.

Muscle models stimulated at higher frequency intervals showed an alteration on early myoblast differentiation. These results suggest an initial impact on muscle biology despite the stimulations were carried for less than 15 minutes. Meanwhile, induction of tetanic contractions for 15 minutes resulted in the generation of local damages and the disruption of the muscular fibres. These damages resembled the structure of second-degree muscle injuries and could only be observed in those regions closer to the graphene microelectrodes after the stimulation.

Conclusions

Graphene microelectrode arrays can generate a localized muscular stimulation in contrast to standard systems. This controlled electrical stimulation does not only allow to recreate natural contractions, but it also induces important biological changes that could help to improve the treatment of complex muscular conditions. The possibility of recreating local tissue damages using this technology opens the opportunity to generate clinically relevant muscle models and explore their rehabilitation *in vitro*.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1480 3D Bioprinting of Bilayered Skin Models Using Dextran-Based Hydrogels.

Rebecca Downs-Ford[1 2], Marco Domingos[1 2], Sarah Cartmell[1 3], David Warde[4]

[1]The Henry Royce Institute, Manchester, United Kingdom; *[2]Department of Mechanical and Aerospace Engineering, School of Engineering, Faculty of Science and Engineering, The University of Manchester, Manchester, United Kingdom*; *[3]Department of Materials, School of Natural Sciences, Faculty of Science and Engineering, The University of Manchester, Manchester, United Kingdom*; *[4]Solventum, Loughborough, United Kingdom*

Introduction/Objectives

Chronic wounds are a global health challenge, with 40-60% failing to heal in 3 months. Limited therapy success emphasises the need for advanced models to improve outcomes. Existing 2D/3D models fail to replicate native skin's complexity, driving demand for reliable, high-throughput alternatives. Typical skin components like collagen and hyaluronic acid face high costs, reproducibility issues, and variability. Additionally, collagen-based bioinks for 3D printing struggle with low viscosity, weak mechanical strength, and crosslinking challenge.

Dextran, a biocompatible polysaccharide, has garnered attention as an alternative for 3D bioprinting due to its favourable rheological properties, gelation behaviour, and biodegradability. However, its potential as a scaffold for full-thickness skin models remains underexplored. This study investigates the use of semi-synthetic dextran-based hydrogels for 3D bioprinting of skin models, aiming to create a reliable, high-throughput platform that closely mimics native skin for chronic wound research and therapeutic testing.

Methods

Dextran was modified with Glycidyl Methacrylate (DexGMA), using a previously established protocol within the group, and characterised using NMR and FTIR spectroscopy to confirm successful modification. Further characterisation was carried out via rheological assessments, along with photo-rheology and mechanical testing of the photo-crosslinkable DexGMA hydrogels. Biocompatability has been assessed through various assays, and function through PCR.

Results

We have successfully incorporated Human Dermal Fibroblasts (HDFn) and various biological components (hyaluronic acid and collagen), into DexGMA-based hydrogels, facilitating the development of a bilayered skin model comprising both dermal and epidermal layers. We have demonstrated favourable crosslinking kinetics and superior printability compared to collagen-based alternatives. Biocompatibility testing confirmed the suitability of DexGMA for the selected human dermal fibroblasts and keratinocytes, with improved viability and morphology in those hydrogels containing HA or Collagen, while immunofluorescence staining, and histology have highlighted matrix formation over the culture period.

Conclusions

This study will present the findings of biocompatibility assays, gene expression and investigations of the interactions of the epidermis and the dermis. We hypothesise that the DexGMA-based bilayered construct will exhibit promising structural and functional characteristics that closely mimics the native dermis, with the opportunity to tune the material to specific wound modelling purposes, positioning it as a viable platform for chronic wound research and therapeutic testing.

We intend to increase the model's complexity further by optimising the incorporation of additional biological components, such as immune cells to improve its physiological relevance with regards to wound healing investigations. Our findings underscore the potential of DexGMA-based hydrogels as a versatile material for 3D bioprinting of full-thickness skin models, offering tuneable mechanical properties, enhanced printability, and biocompatibility. These advances may facilitate the development of more reliable, reproducible models for chronic wound therapy and contribute to the reduction of animal testing in preclinical studies.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1490 Generation of artificial hyaline cartilage substitutes by microtissues and fibrin-agarose hydrogels. David Sánchez-Porras[1 2], Miguel Etayo-Escanilla[1 2], Paula Ávila-Fernández[1 2], Fabiola Bermejo-Casares[1 2], Olimpia Ortiz-Arrabal[1 2], Jesús Chato-Astrain[1 2], Fernando Campos[1 2], Antonio Campos[1 2], Víctor Carriel[1 2]

[1]Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; *[2]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*

Introduction/Objectives

Hyaline cartilage is susceptible to being affected by multiple pathologies, especially, degenerative or traumatic diseases due to their elevate prevalence in society. Cartilaginous tissues have a reduced intrinsic capacity for regeneration because of their special conditions, such as an avascular extracellular matrix (ECM) and reduced metabolic activity of adult chondrocytes. For this reason, current therapies have not achieved effective long-term results, especially in joint injuries [1]. The main limitations of current therapies are related to the dedifferentiation processes associated with in vitro chondrocyte culture and poor biomimetic substitutes consisting of type I collagen scaffolds, which can induce fibrocartilage formation. In this sense, our aim was to generate a more biomimetic cartilage artificial substitute by combining cartilaginous microtissues (MTs) with fibrin-agarose biomaterials, which have been clinically used for the treatment of skin, palate or corneal defects with success [2].

Methods

Hyaline MTs were generated in chondrogenic conditions during using the agarose-microchip technique [3]. After 7 days of development, MTs were harvested, encapsulated in fibrin-agarose hydrogels and evaluated for 28 days by Live/Dead assay, WST-1 assay and histological methods. In this way, the

differences in behavior between MTs and dispersed hyaline chondrocytes encapsulated in fibrin-agarose (DC) were analyzed.

Results

During 28 days of development, MTs and dispersed chondrocyte substitutes showed elevated viability rates and an increasing metabolic activity profile. Histological results revealed the colonization capacity of chondrocyte-forming microtissues in the biomaterial and a superior presence of the principal hyaline cartilage ECM molecules, such as collagen type II and proteoglycans (aggrecan and biglycan), in MT compared to DC substitutes. Moreover, the synthesis of collagen type IV, an essential element of pericellular matrix of native chondrocytes, was higher and earlier in time in MT cartilaginous constructs.

Conclusions

Our experiments demonstrated for the first time the feasibility of combining chondrocyte-derived microtissues with fibrin-agarose hydrogels to generate enhanced ECM cartilaginous substitutes compared to the application of dispersed chondrocytes. Nevertheless, further in vitro and in vivo analyses are needed to elucidate the regenerative potential of these substitutes.

Acknowledgements: This research was supported by grant CS PI-0257-2017 from Consejería de Salud y Consumo, Junta de Andalucía, Spain and CTS-115 (Tissue Engineering Group).

References

[1] Fox S., et al. Sports health 1.6 (2009): 461-468.

[2] Sánchez-Porras D., et al. (2021). Biomedicines 9(3), 292.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1492 Development and characterization of an ultra-rapid decellularization protocol for human limbal substitutes generated by tissue engineering for use in limbal stem cell deficiency.

Jesús Chato-Astrain[1], Paula Ávila-Fernández[1], David Sánchez-Porras[1], Miguel Etayo-Escanilla[1], Fabiola Bermejo-Casares[1], Carmen González-Gallardo[2], Fernando Campos[1], Miguel Ángel Martín-Piedra[1], Óscar Darío García-García[1], Miguel Alaminos[1]

[1]*University of Granada, Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*; [2]*Division of Ophthalmology, University Hospital Clínico San Cecilio, Granada, Spain*

Introduction/Objectives

Limbal Stem Cell Deficiency (LSCD) is associated with a severe alteration of corneal turnover, leading to severe vision loss and reduced quality of life. Current treatments often face challenges, such as donor site morbidity or prolonged immunosuppressive therapy. This study aims to develop and evaluate a novel ultra-rapid decellularization protocol to generate human limbal substitutes potentially useful in cell-based therapies for LSCD. Ultra-rapid decellularization protocols lasting only 8 hours were developed and evaluated in comparison to traditional longer-lasting protocols, and other rapid protocols previously published.

Methods

Seven detergent-based decellularization protocols (from P1 to P7) were applied to the limbal tissue, including four previously published protocols developed by our research group showing usefulness in porcine limbal decellularization (P1-P4) [1], two novel protocols based on a combination of chemical and enzymatic agents (P5-P6) and a published protocol used to decellularize the human limbus in 24h (P7) [2]. Decellularization efficiency, ECM preservation, and recellularization potential were assessed using histological, histochemical, and immunostaining analysis methods.

Results

Previously published P1-P4 protocols were less efficient for limbal decellularization, with longer processing times and reduced ECM preservation. The newly developed P6 protocol demonstrated superior decellularization efficiency and ECM preservation, outperforming P1-P4. Additionally, P6 maintained critical ECM components, such as proteoglycans and glycosaminoglycans better than longer protocols and exhibited enhanced biocompatibility. Recellularization studies showed cell adhesion, proliferation, and partial differentiation, with MSCs expressing key corneal markers like cytokeratin 3 and crystallin-αA.

Conclusions

The novel ultra rapid protocol P6 was able to improve the results of previously developed protocol, resulting in a significant advancement in decellularization technology, combining fast processing with superior ECM preservation and cellular biocompatibility. As compared with the rapid protocol P7, this novel protocol was able to reduce decellularization time while preserving efficiency and ECM preservation. These findings suggest that P6 could be used as a promising candidate for the generation of limbal substitutes for LSCD therapy.

This work was supported by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities, grants FIS PI23/00335 and ICI21/00010 (NANOULCOR). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

References

[1] Sánchez-Porras, D., et al. (2021). Generation of a Biomimetic Substitute of the Corneal Limbus Using Decellularized Scaffolds. *Pharmaceutics* 13, 1718. doi: 10.3390/pharmaceutics13101718

[2] Polisetti, N., et al. (2021). A decellularized human corneal scaffold for anterior corneal surface reconstruction. *Sci Rep* 11, 2992. doi: 10.1038/s41598-021-82678-3

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1498 In vitro characterization of novel hybrid nerve conduits based on 3D printed PLLA tubular mesh and fibrin-based hydrogels. Miguel Etayo-Escanilla[1 2], David Sánchez-Porras[1 2], Paula Ávila-Fernández[1 2], Óscar Darío García-García[1 2], Jesús Chato-Astrain[1 2], Fernando Campos[1 2], Elías Khoury-Martín[1], Sandra Vieira[3], Marta Pegueroles[4], Víctor Carriel[1 2]

[1]Department of Histology & Tissue Engineering Group, University of Granada., Granada, Spain; *[2]Instituto de Investigación Biosanitaria ibs.GRANADA., Granada, Spain*; *[3]Department of Medical Sciences, Institute of Biomedicine (iBiMED), University of Aveiro, 3810-193, Aveiro, Portugal*; *[4]Biomaterials, Biomechanics and Tissue Engineering Group. Dept. Material Science and Engineering, Universitat Politècnica de Catalunya, EEBE, Barcelona, Spain*

Introduction/Objectives

Peripheral nerve injuries (PNIs) are prevalent conditions that significantly impair patients' quality of life, with substantial functional and psychological consequences. Recent studies have exhibited the potential use of 3D-printed nerve conduits in the treatment of PNI [1]. Therefore, this technology can be applied to generate novel nerve conduits with better biomechanical and structural properties than conventional techniques [2]. In this sense, the aim of this study was to generate PLLA tubular meshes and functionalize them with different fibrin-based hydrogels, in order to improve their biological properties and generate novel hybrid conduits (HCs) for peripheral nerve repair.

Methods

PLLA tubular meshes (20x3 mm) were solvent-cast direct-3D printed (PLLA group) and coated with fibrin (PLLA-F group), fibrin-agarose (PLLA-FA group) and 0.1% genipin-crosslinked FA (PLLA-FA-G group) hydrogels. The generated HCs were processed and analysed by scanning electron microscopy and

compression tests were applied to determine their biomechanical properties. Moreover, the HCs biological properties were evaluated through the culture of human neural SH-SY-5Y cells on the conduit surfaces, and cell viability was inferred using Resazurin (on days 1 and 3) and Live/Dead assays (on day 3).

Results

The biomechanical analysis revealed comparable load values at 20% and 40% diameter compression across all groups, with the coated groups exhibiting superior viscoelastic properties, as indicated by the Young's modulus. Concerning the metabolic and viability results, the application of fibrin-derived hydrogels showed an improvement in these values as compared to uncoated PLLA. These results were especially superior in the PLLA-FA-G group as determined by resazurin and Live/Dead assays.

Conclusions

The functionalization (coating) with fibrin-based hydrogels, especially FA and FA-G, resulted in a clear improvement of the PLLA conduits biological properties in vitro while improving their viscoelastic properties. Furthermore, in vivo biocompatibility and nerve repair studies are still needed to determine their potential use in peripheral nerve repair.

Fundings

This work was financed by the Spanish "Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica" (I+D+i) of the Ministry of Science and Innovation of Spain (Instituto de Salud Carlos III) and co-financed by FEDER funds (European Union) Grant FIS PI23/00337; "Proyectos de colaboración público-privada, Plan de Investigación Científica, Técnica y de innovación 2021–2023 (MCIN/AEI/10.13039/501100011033 and European Union-NextGenerationEU/PRTR) (Grant CPP2021-009070). MEE by FPU Fellowship Grant FPU21/06183 of the Spanish Ministry of Universities.

References

[1] Kai Liu, et al. AdvSci, 2022;9(12) e2103875

[2] Vijayavenkatamaran S. Acta Biomater. 2020;106:54-69

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1502 Synergistic Integration of Functionalized Scaffolds and a Novel Bioreactor for Enhanced Cartilage Regeneration. Daniel Martinez-Moreno[1], Antonio Callejas[2], Gema Jiménez[1], Patricia Galvez-Martin[3], Guillermo Rus[2], Elena López-Ruiz[4], Juan Antonio Marchal [1]

[1]University of Granada, Faculty of Medicine, Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; *[2]University of Granada, Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*; *[3]Bioiberica SAU, Barcelona, Spain*; *[4]Univesity of Jaén, BioFabi3D Lab, Department of Health Siciences, Jaén, Spain*

Introduction/Objectives

Osteoarthritis (OA) presents a significant socio-economic challenge due to the limited regenerative capacity of articular cartilage. Current tissue engineering (TE) strategies aim to develop functional cartilage-like tissues that can restore joint function effectively. This study combines a novel low-shear stress-based bioreactor with advanced functionalized scaffolds to enhance chondrogenesis. Scaffolds made from 1,4-butanediol thermoplastic polyurethane (bTPUe), functionalized with pyrene butyric acid (PBA), were used to provide a mechanically and biologically conducive environment for infrapatellar fat pad mesenchymal stem cells (IPFP-MSCs).

Methods

The bioreactor, designed to generate viscous shear stress via controlled flow perfusion, was integrated with a real-time low-intensity pulsed ultrasound (LIPUS) monitoring system. The bTPUe scaffolds were

functionalized using PBA to improve cell adhesion and proliferation. IPFP-MSCs were seeded on these scaffolds and cultured for 21 days under perfusion. Chondrogenic differentiation was evaluated through metabolic activity assays, extracellular matrix (ECM) analysis, and immunofluorescence. Real-time tissue evolution was monitored using a finite element model (FEM) to interpret ultrasound signal propagation.

Results

PBA-functionalized bTPUe scaffolds exhibited superior cell adhesion, proliferation, and ECM deposition compared to unmodified scaffolds. The bioreactor system successfully induced chondrogenic differentiation without the need for pre-conditioning media, as demonstrated by significant upregulation of chondrogenic markers (Sox9, Col2a, and aggrecan). Additionally, LIPUS monitoring provided continuous, non-invasive insights into tissue development, correlating scaffold mechanical changes with cellular activity. FEM analysis validated ultrasound signal variations as indicators of cartilage maturation.

Conclusions

This study demonstrates the synergistic effect of functionalized scaffolds and mechanical stimulation within a bioreactor system to enhance cartilage regeneration. The integration of real-time ultrasound monitoring with scaffold-based chondrogenesis represents a significant advance in tissue engineering for osteoarthritis treatment, offering a scalable, efficient approach for developing bioengineered cartilage substitutes.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1503 Fabrication of biomimetic bilayered human urethra and skin tissue substitutes using functionalization with bioactive molecules of natural origin. Miguel Etayo-Escanilla [1], Olimpia Ortiz-Arrabal[1], Paula Ávila-Fernández[1], David Sánchez-Porras[1], Ingrid Garzón[1], Maria del Carmen Sánchez-Quevedo[1], María-Dolores Mesa-García[2], Carolina Gómez-Llorente[2], Miguel Alaminos[1], Jesús Chato-Astrain[1]

[1]*University of Granada, Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*; [2]*Department of Biochemistry and Molecular Biology II, School of Pharmacy, Campus Universitario de Cartuja, Institute of Nutrition and Food Technology "José Mataix", Biomedical Research Center, University of Granada, Granada, Spain*

Introduction/Objectives: Generation of bilayered tissue substitutes such as the human urethra and skin is challenging, since an efficient induction of epithelial and stromal differentiation cannot be normally achieved in culture [1]. One of the possible approaches favoring *ex vivo* differentiation is the use of bioactive molecules extracted from natural sources, including the virgin olive oil. This product demonstrated to be very rich in bioactive components, and can be used to favor tissue regeneration *in vivo* [2]. The objective of this work is to determine if bioactive components of the olive oil can be used to functionalize human tissues to generate more biomimetic substitutes of the human urethra and skin.

Methods: Human stromal and epithelial cells were isolated, kept in culture, and used to generate bilayered human tissue substitutes with a fibrin-agarose stroma and a stratified epithelium on top. Bilayered tissues were functionalized with phenolic extract (PE) and oleuropein (OL) in the biomaterial and culture medium. Tissues without these components were used as controls (CTR). CTR, PE and OL bilayered tissues were cultured for 12 days, and the epithelial and the stromal layers were analyzed to identify relevant markers of differentiation.

Results: Tissues functionalized with PE showed an increase in epithelial development as compared to CTR, although tissues functionalized with OL were lower than CTR. At the epithelial layer, we found that PE samples were able to express higher amounts of cytokeratins by pancytokeratin expression analysis, than

CTR, but no difference was found between OL and CTR. Cell proliferation as determined by PCNA immunohistochemistry revealed that epithelial cells were able to actively proliferate in the three studied groups (PE, OL and CTR). When the stromal layer was analyzed, we found very low expression of the typical markers of the stromal extracellular matrix in PE, OL and CTR.

Conclusions: These results suggest that certain components of the virgin olive oil, especially PE, were able to contribute to a more biomimetic development of the epithelial layer of bioengineered bilayered substitutes, without altering the intrinsic proliferation capability of these cells kept in culture. However, the effects of functionalization were less significant regarding stromal differentiation. These findings drive us to hypothesize that the use of olive oil components could improve current biofabrication methods, allowing the generation of more biomimetic substitutes of the human urethra and skin by tissue engineering.

This work was supported by the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), Grant FIS PI22/00059. Supported by Plan Propio de Investigación y Transferencia de la Universidad de Granada (grant C-CTS-032-UGR23). Cofinanced by the European Regional Development Fund (FEDER/ERDF) through the “Una manera de hacer Europa” program, European Union.

References

[1] Garzón, I., et al. (2009). In vitro and in vivo cytokeratin patterns of expression in bioengineered human periodontal mucosa. *J. Periodont. Res.* 44, 588–597

[2] Donato-Trancoso, A., et al. (2023). Dual effects of extra virgin olive oil in acute wounds. *Wound Repair Regen* 31, 338–348

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1516 Evaluation of different recellularization techniques for peripheral nerve allografts. David Sánchez-Porras[1 2], Giorgia Ongis[1], Paula Ávila-Fernández[1 2], Miguel Etayo-Escanilla[1 2], Miguel Alaminos[1 2], Jesús Chato-Astrain[1 2], Fernando Campos[1 2], Óscar Darío García-García[1 2], Víctor Carriel[1 2]

[1]Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; *[2]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*

Introduction/Objectives

Peripheral nerve injuries (PNIs) represent a significant challenge in clinical practice due to their impact on motor, sensory and autonomic functions. In the last years, decellularized nerve allografts (DNA) have garnered attention due to the promising experimental results obtained in the repair of PNIs in rodents [1]. However, they have been reported inferior results than the gold-standard, the nerve autografts, in part, because of the lack of cells, which are determinant for nerve regeneration [2]. Thus, seeding cells onto decellularized scaffolds could solve this limitation, but the process can also be challenging. Therefore, this study aims to compare different recellularization methods to functionalize DNA.

Methods

Wistar rat sciatic nerves were decellularized by previously described protocols [1]. After the efficacy evaluation of decellularization, nerve conduits were recellularized with adipose-derived stem cell (ADSC) by using two techniques: ADSC injected alone and ADSC incorporated in combination with fibrin hydrogels. Substitutes were maintained in standard culture conditions and cell viability and histological analyses were carried out at 3, 10, and 21 days.

Results

Evaluation of the decellularization process shows an optimal removal of nuclear content with a decrease in glycoproteins and proteoglycans. However, the nerve microarchitecture and the collagen network were

both well-preserved. Afterwards, recellularization studies using different techniques demonstrated the viability of injected ADSC at all timepoints, but with rounded morphology. In addition, the incorporation of ADSC with fibrin hydrogel into DNA showed high cell viability and cells adopted clear signs of elongation at 10 days of culture. Interestingly, cell migration from nerve surface into nerve core was observed in both strategies after 21 days of culture, this phenomenon being more noticeable when fibrin hydrogel was used as a carrier of ADSC.

Conclusions

In this study, we have successfully generated cellular nerve substitutes by direct injection or with fibrin as a carrier, which not only enhances the control of the cell seeding providing cell adhesion but also facilitates proliferation, morphological changes and migration due to the presence of natural adhesion substrate and biocompatible cues. However, further experiments are still required to prove the therapeutic efficacy of these novel cellular DNA.

Fundings: This work was financed by the Spanish “Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica” (I+D+i) of the Ministry of Science and Innovation of Spain (Instituto de Salud Carlos III) and co-financed by FEDER funds (European Union) Grant FIS PI23/00337; “Proyectos de colaboración público-privada, Plan de Investigación Científica, Técnica y de innovación 2021–2023 (MCIN/AEI/10.13039/501100011033 and European Union-NextGenerationEU/PRTR) (Grant CPP2021-009070). Universidad de Granada “Ayudas del plan propio UGR 2022, Plan propio de investigación y transferencia” grant number PPJIA2022-19. MEE by FPU Fellowship Grant FPU21/06183 of the Spanish Ministry of Universities.

References

[1] García-García D., et al. (2023. *Front Bioeng Biotechnol*. 2023 Mar 30;11:1162684

[2] Carriel, V., et al. (2014). *Expert review of neurotherapeutics*, *14*(3), 301–318

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1523 Electroactive nanofibrous membranes as scaffolds for neural regeneration.

Konstantinos Loukelis[1], Stefanos-Ioannis Kaplanis[1], Dimitris Spyridakos[1], Domna Karagogeos[2], Maria Chatzinikolaidou[3]

[1]University of Crete, Heraklion, Greece; *[2]University of Crete, FORTH-IMBB, Heraklion, Greece*; *[3]University of Crete, Foundation for Research and Technology Hellas, Heraklion, Greece*

Introduction/Objectives

Neural network’s complex physiology often proves to be the target of various pathologies, either as the result of congenital diseases or due to external traumas. Neural tissue has low endogenous regenerative capacity, while extensive damage can lead to irreparable disruption of its base metabolic activities and thus severely hamper the patient’s quality of life. To overcome such pathologies, neural tissue engineering strives for the development of novel medicinal platforms that expediate healing of native tissue, by eliciting specific biofunctional cues when situated in close proximity to the damaged area. In respect to the outmost importance of electrical conductivity on the physiological function of nerves, we developed electrospun fibrous membranes, coated with different concentrations of the biocompatible, intrinsically conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT), as a platform for neural tissue reconstitution. PEDOT has been used as blend with polystyrene sulfonate (PSS) to facilitate its water solubility.

Methods

Electrospun scaffolds comprising gelatin and polyvinyl(alcohol) (PVA) have been fabricated and coated with various PEDOT:PSS concentrations. The material characterization of the resulting functional scaffolds,

in terms of chemical integrity, morphology, thermal stability and conductivity has been performed. We have examined (i) the in vitro biocompatibility and differentiation potential of two key regulator glial cell types for remyelination, microglial and oligodendrocyte cells, and (ii) the antibacterial effect of the PEDOT:PSS coated scaffolds.

Results

The data illustrated the antibacterial properties of the coated nanofibrous membranes against Gram positive and Gram negative bacterial strains. Data verified the biocompatible nature of the scaffolds, since they allow for microglial and oligodendrocytes cell adhesion. We found that the electroactive nanofibrous scaffolds promoted rapid proliferation of oligodendrocyte precursor cells (OPCs) and their differentiation to mature oligodendrocytes that are responsible for the production of myelin. In addition, the data revealed that the scaffolds have not reduced the reactivity of microglia and the release of pro-inflammatory cytokines from these cells, suggesting that the scaffolds did not possess anti-inflammatory properties.

Conclusions

The electroactive electrospun nanofibrous scaffolds coated with PEDOT:PSS display strong anti-bacterial properties, while they are capable of affecting the physiology of oligodendrocytes, indicating that they might promote myelin repair. However, their beneficial effect on oligodendrocytes is not in line with their inability to reduce the inflammatory response of microglia, since inflammation is a crucial process that inhibits myelination and remyelination processes. Next goal is the investigation of the in vivo functionality of the scaffolds, by employing mice models to illustrate their efficacy as regenerative implants for different neural related anatomical sites.

Topic: Tissue Engineering

Subtopic: Tissue modelling and repair

Type: Poster presentation

TERMIS25_1603 Extracellular Vesicles embedded 3D printed scaffold as cell-free therapeutic for bone regeneration and repair. Aditi Bharadwaj[1], Ashutosh Bagde[2]

[1]DST INSPIRE Phd Fellow, Datta Meghe Institute of Higher Education and Research, Wardha, India; *[2]Add'l Director Bionest, Research Scientist, Datta Meghe Institute of Higher Education and Research, Wardha, India*

Introduction/Objectives

Bone diseases are common yet increasing in prevalence mainly due to trauma, fracture and age-related factor worldwide, ultimately affecting the quality of life. The existing therapies has been using autografts, xenografts and allograft along with cell therapy impose the disadvantages of immune rejection and lack of vascularization. Whereas the need to explore beyond the cell, moving towards the cell-free therapeutics using extracellular vesicles are the next generation therapies. These EVs do possess even the higher therapeutic potential than their parent stem cells. Our research focuses on the repair and regeneration efficiency of EV therapy for the bone tissue differentiation with the 3D Bio printed scaffold with natural and synthetic biomaterials. Among the Extracellular vesicles especially exosome plays leading role in therapeutic application in regenerative medicine for bone, skin, cartilage, etc. These vesicles are nano sized, immunomodulator and transmitting signals between neighboring cells via paracrine or endocrine mechanism making them a promising tool for regeneration and rejuvenation. Therefore, our objective was to use exosome as cell-free therapy to treat the bone defects with the 3D printed fabricated scaffold for the effective regenerative effect. We aim to develop the bio composite ink of β-Tricalcium Phosphatase, nanohydroxyapatite, polycaprolactone and chitosan for extrusion-based 3D bioprinting.

Methods

In this study, we used human adipose derived stem cells hASCs derived exosome isolated via ultracentrifugation method. On the other hand, the β-TCP/nHA/PCL/Chitosan ink has been synthesized for extrusion-based 3D bioprinting followed by UV crosslinking. The characterized exosome as per the MISEV guidelines were further reinforced with the 3D printed scaffold. The structural property was studied using scanning electron microscopy. The exosome encapsulation, cytocompatibility and osteogenic effects of both the scaffold and EV reinforced scaffold were also studied with the osteoblast cells, and further validated the enhanced regenerative efficacy of EVs.

Results

We have characterized the structure, size, concentration, surface marker analysis as per the MISEV guidelines for the EVs to identify them as exosome. Also, systematically characterized the functional and phase analysis of the synthesized ink composite. The ink has been validated for extrusion-based printing with the rheological evaluation defining the desired shear thinning behavior. The surface morphology, dissolution kinetics, biocompatibility and osteogenic potential of the EV reinforced scaffold has been done. The EV reinforced scaffold increased the osteoblast differentiation, proliferation due to upregulated osteogenic gene expression via activating signaling pathways.

Conclusions

This study highlights that the 3D-printed β-TCP/nHA/PCL/Chitosan scaffold, reinforced with exosomes, has significant potential to enhance and accelerate the bone healing and repair mechanism.

Topic: Tissue-Specific Focus

Subtopic: Adipose

Type: Poster presentation

TERMIS25_547 Role of defined in vitro human adipose tissue models in macrophage-driven inflammation and body mass index-based disease modeling. Sophia Nowakowski[1] [2], Svenja Nellinger[2], Franziska Albrecht[2], Petra Kluger[2]

[1]*Hohenheim University, Stuttgart, Germany*; [2]*Reutlingen University, Reutlingen, Germany*

Introduction/Objectives

Adipose tissue dysfunction is characterized by excess adipose tissue and is associated with systemic inflammation and metabolic dysregulation, leading to conditions such as type 2 diabetes and cardiovascular disease. This inflammation drives insulin resistance and metabolic impairment, with macrophages playing a central role by releasing pro-inflammatory cytokines that exacerbate adipocyte dysfunction. Here, we investigate two in vitro models of human adipose tissue disease to elucidate these mechanisms: (1) an inflamed adipose tissue model with macrophages as immune components and (2) an obesity model using adipose tissue from donors across a range of body mass indices (BMI). By examining inflammatory and metabolic responses, this study aims to enhance understanding of obesity-driven adipose tissue changes, allowing for better disease modeling and therapeutic testing.

Methods

For the inflamed adipose tissue model, human mature adipocytes (ACs) were encapsulated in gellan gum and co-cultured with MM6 or THP-1 macrophages in a defined medium to simulate an inflammatory environment. Macrophages were polarized towards a pro-inflammatory phenotype using lipopolysaccharides (LPS) and phorbol-12-myristate-13-acetate (PMA) to mimic immune-mediated inflammation commonly seen in obesity. In the obesity model, human adipose-derived stem cells (hASCs) were obtained from individuals with different BMIs (normal, overweight, and obese) and differentiated into adipocytes in a defined medium without immune cells, allowing for a comparison of cellular responses across BMI groups. Inflammatory cytokine profiles (e.g., TNF-α, IL-6, IL-8, IL-1β) were assessed by enzyme-linked immunosorbent assay (ELISA). Metabolic functions were analyzed via stimulated glucose uptake and

glycerol assay. Lipid content and lipid vacuole quality were analyzed using BODIPY and perilipin A staining.

Results

The inflamed adipose tissue model with ACs and macrophages showed significantly elevated levels of pro-inflammatory cytokines upon stimulation compared to unstimulated controls. This demonstrated an immune cell-dependent inflammatory response with different cytokine profiles for the co-culture with either MM6 or THP-1 macrophages compared to monoculture. In the defined BMI-based obesity model, differentiated hASCs from higher BMI donors showed altered metabolic activity patterns, with impaired glucose uptake and lipolysis compared to lower BMI groups.

Conclusions

These findings demonstrate that the inflamed adipose tissue model with pro-inflammatory-polarized macrophages provides a robust in vitro approach to simulate the inflammatory milieu associated with obesity. On the other hand, the BMI-based model allows for examining metabolic alterations arising intrinsically within adipocytes as BMI increases, thus offering insights into obesity's underlying molecular changes independent of acute immune activation. The macrophage-inflamed model may better mimic acute inflammatory states of adipose tissue in obesity, while the BMI-based model reveals underlying adipose tissue dysfunction directly associated with adipocytes. In summary, these models provide complementary platforms for studying obesity-related adipose tissue dysfunction and testing anti-inflammatory and lipolytic therapies for treating obesity and related metabolic diseases.

Topic: Tissue-Specific Focus

Subtopic: Adipose

Type: Poster presentation

TERMIS25_1072 Characterizing Adipose-Derived Mesenchymal Stem Cells in 2D and 3D Models. Zahra Kamyab[1 2], Karin Stenkula[3], Gunnar Mellgren[1 2], Johan Fernø[1 2]

[1]Hormone Laboratory, Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway; *[2]Mohn Nutrition Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway*; *[3]Department of Experimental Medical Science, Lund University, Lund, Sweden*

Introduction/Objectives

Adipose tissue is an important source of mesenchymal stem cells (MSCs). In vitro studies on these multipotent adult stem cells provide insights into the molecular regulation of adipocyte development as well as their role in obesity and metabolic conditions. Research has shown that adipose-derived MSCs include subpopulations capable of differentiating into mature adipocytes with varying metabolic properties. Recent advancements in self-organized 3D culture models offer an organotypic microenvironment, enhancing in vitro MSC differentiation into unilocular adipocytes compared to monolayer cultures. However, it remains unclear how well current in vitro models reflect the inherent MSC heterogeneity of native tissue. This study aims to examine the cellular composition of adipose-derived MSCs in both scaffold-free and vascularized 3D spheroids compared to 2D cultures, with a focus on MSC proliferation and adipogenic induction.

Methods

Stromal vascular fraction (SVF) was isolated from human white adipose tissue samples obtained during elective liposuction procedures. The cells were cultured in 2D monolayer and as 3D scaffold-free spheroids or Matrigel-embedded vascularized spheroids for 6–10 days. Following adipogenic stimulation, the cells were maintained for up to 30 days in the adipocyte differentiation medium. To assess the cellular heterogeneity, we performed flow cytometry analysis of common stem cell markers, including CD90, CD73, CD29, and several subtype-specific markers, like CD34, CD26 (DPP4), and CD54 (ICAM-1), post-

expansion and after three days of adipogenic induction. Fluorescent staining and confocal microscopy were used to investigate cellular morphology and lipid content of the in vitro-differentiated adipocytes.

Results

High-dimensional screening of surface proteins revealed that while MSC proliferation correlated with higher expression levels of common stem cell markers across all culture conditions, each in vitro model exhibited distinct profiles of surface markers expression. Additional analyses identified differentially enriched MSC clusters among the experimental conditions. Adipogenic induction led to a coordinated alteration in surface markers expression, yet the overall distinction in cell surface phenotypes between 2D and 3D culture systems appeared consistent. Expectedly, adipocytes differentiated in 3D models developed larger lipid droplets than those in 2D cultures.

Conclusions

Our findings support that 3D differentiation of adipocytes can mimic the in vivo adipocyte phenotype, particularly with respect to lipid droplet formation. However, our study indicates that adipose-derived MSCs adopt culture-specific cellular profiles, which may be preserved during adipogenic differentiation. Additional transcriptomic analyses provide further insight into MSC characteristics in current culture systems, optimizing model selection for future translational applications.

Topic: Tissue-Specific Focus

Subtopic: Angiogenesis, vasculogenesis

Type: Poster presentation

TERMIS25_624 Developing an angiogenic periosteum substitute to improve delayed bone healing. Caitlin Ryan[1], Gwendolen Reilly[1], Frederik Claeyssens[1]

[1]*University of Sheffield, Sheffield, United Kingdom*

Introduction/Objectives

Periosteal grafts can be used to treat delayed bone healing injuries, namely critical size defects and non-unions. However, this treatment is uncommon despite the fact that excellent union rates have been reported. This is due to numerous limitations including the difficulty of graft harvest and limited available graft size. Research surrounding artificial periosteum substitutes is growing, and synthetic membranes are being developed to replace periosteal grafts and provide a tailorable solution with fewer associated complications. However, developed membranes are often very complex and whilst having good results, are not realistically translatable to a clinical setting. The present work demonstrates a simple, easy-to-handle periosteum substitute made using readily available laboratory equipment which addresses several common barriers to clinical translation.

Methods

The periosteum substitute was made of a 50:50 blend of the synthetic and biodegradable polymers polycaprolactone methacrylate (PCLM) and poly(glycerol sebacate) methacrylate (PGSM). The blend was used to make photocurable polymer high internal phase emulsions (PolyHIPEs) which, once exposed to UV light, formed structures with multiscale porosity. The success of periosteal grafts is in part owed to their ability to revascularise an injury site. Hence, to encourage vascularisation of the periosteum substitute, and subsequent revascularisation of a bone injury site, the membrane was loaded with a highly concentrated solution of the angiogenic sugar 2-deoxy-D-ribose (2dDr). Membranes were seeded with periosteal-typical cells to assess their response, and their angiogenic potential was assessed using an *in vivo* chick chorioallantoic membrane (CAM) assay.

Results

Scanning electron microscopy revealed that the membranes had large, well-interconnected porosity with average pore and window diameters of 69.2 μm and 15.5 μm respectively. The multiscale porosity of the

periosteal membrane makes it ideal for supporting endogenous cell migration and nutrient transfusion. Bial's assay demonstrated that despite using a straightforward loading method, the release of 2dDr from the membrane was prolonged with 50%, 78%, and 99% of 2dDr being released on day 1, day 4, and day 8 respectively. Membranes loaded with a 5% wt 2dDr solution had no significant negative impact on the growth of osteoblasts or mesenchymal stem cells, two major cell populations within the periosteum. *In vivo* assessment of blood vessel formation demonstrated that these membranes also caused a significant increase in blood vessel density surrounding the implanted membrane compared to non-2dDr controls. The membranes were well integrated into the surrounding tissue, and blood vessels could clearly be seen growing into the membranes.

Conclusions

This study demonstrated that periosteum substitutes do not need to be complex to facilitate revascularisation of a delayed healing bone injury. Furthermore, early *in vitro* and *in vivo* assessments of osteogenesis indicate this polyHIPE membrane is a promising material for bone repair.

Topic: Tissue-Specific Focus

Subtopic: Angiogenesis, vasculogenesis

Type: Poster presentation

TERMIS25_1174 Molecular mechanisms of vessel morphogenesis. Maria Markou[1], Elena Rakovoliou[2], Eleni Bagli[1], Sofia Bellou[3], Theodore Fotsis[2], Carol Murphy[1]

[1]Biomedical Research Institute - FORTH, Ioannina, Greece; *[2]Biomedical Research Institute - FORTH, Laboratory of Biological Chemistry, Medical Faculty, School of Health Sciences, University of Ioannina, Ioannina, Greece*; *[3]Biomedical Research Institute - FORTH, Confocal Laser Scanning Microscopy unit, Network of Research Supporting Laboratories, University of Ioannina, Ioannina, Greece*

Introduction/Objectives

Regenerative Medicine (RM), based on the immense regenerative capacity of stem cells, is considered the therapy of the future. However, there are still issues that need to be addressed before translation in the clinic. One important factor in the success of RM is the speed by which the transplanted cells are vascularised by the host. Delayed vascularisation of the implant results in low cell survival rates. Thus, ideally the tissue engineered construct should be already vascularised *in vitro* prior to implantation *in vivo*. We have already initiated a programme for vascular engineering based on reprogramming human fibroblasts to generate human induced pluripotent stem cells (hiPSCs) and subsequent differentiation of hiPSCs or human embryonic stem cells (hESCs) to endothelial (ECs)[1] and mural cells (MCs)[2]. VEGF is the main growth factor responsible for the endothelial cell commitment during vasculogenesis and angiogenesis. Currently there is a lack of data concerning the VEGF-induced signalling cascades that differentiate/commit mesodermal intermediates to vascular progenitor cells VPCs and then to ECs (vasculogenesis). In addition to VEGF, several other parameters affect vasculogenesis *in vivo*, including the presence of MCs and flow.

Methods

For ECs generation, we differentiated human pluripotent stem cells (hPSCs) via mesodermal intermediates, to vascular progenitor cells (VPCs) and then to ECs[1]. To explore the VEGF-A induced signalling responsible for the transition of mesodermal cells to VPCs, first we sought to identify the mesodermal cell population (Day 3 of the differentiation process), responsive to VEGF-A stimulation, which gives rise to VPCs. FACS sorting of D3 mesodermal cells following *in vivo* labeling with a non-functional anti-VEGFR-2-Alexa488 antibody prior to induction with VEGF-A was performed for this purpose. hPSCs were differentiated to MCs via a robust protocol in feeder free conditions[2]. In addition, a microfluidic system has been established to mimic blood flow of the vessels.

Results

We have established that a subset of D3 mesodermal cells expressing high levels of VEGFR-2 give rise to VPCs following VEGF induction. These cells have been isolated and scATAC seq, scRNAseq and phosphoproteomic analysis are ongoing.

In addition to the role of VEGF, we are addressing the role of flow and MCs involvement on the differentiation efficiency of mesodermal cells to VPCs and ECs maturation, using microfluidic systems to recapitulate the early stages of vasculogenesis.

Conclusions

We have established an *in vitro* model of vasculogenesis by differentiating hPSCs to mesodermal intermediates and then to VPCs and ECs. To further refine the model, we have incorporated MCs and flow using a microfluidic system. VEGF signalling cascades during vasculogenesis are ongoing using scATACseq, scRNAseq, and phosphoproteomic analysis and bioinformatic analysis.

* The research project is implemented in the framework of H.F.R.I call “Basic research Financing (Horizontal support of all Sciences)” under the National Recovery and Resilience Plan “Greece 2.0” funded by the European Union-Next Generation EU (H.F.R.I. Project Number:15009).

REFERENCES

[1] KC Tsolis et al., J Proteome Res **15**(6),1995 (2016)

[2]M Markou et al., Front Bioeng Biotechnol **8**,278 (2020)

Topic: Submit to SYMPOSIUM

Subtopic: Vascularization for Tissue Engineering and Regenerative Medicine

Type: Poster presentation

TERMIS25_1292 Combination of electrospinning and a cell laden collagen hydrogel enables the generation of a central vessel potentially suited for generation of perfusable tissue engineered constructs.

Daria Wehlage[1], Andrea Ehrmann[2], Birgit Andrée[1]

[1]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiothoracic, Transplantation and Vascular Surgery, MHH, Hannover Medical School, Hannover, Germany; *[2]Faculty of Engineering and Mathematics, Bielefeld University of Applied Sciences and Arts, Bielefeld, Germany*

Introduction/Objectives

Tissue engineering (TE) is a promising technique for reconstruction of failing tissues and organs. The major obstacle in TE is the nourishment of cells in dense tissues of clinical relevant dimensions. Therefore, the implementation of a perfusable vascular system lined with endothelial cells (ECs) is crucial, as this is an integral part of every native tissue. Vascularization is not only indispensable for the nourishment of cells with nutrients and oxygen but also for application of drugs. Although substantial progress has been achieved in the last decades, the generation of a functional vascular tree with small arteries for surgical anastomosis and a connection to capillaries is still a major hurdle in tissue construction. In this study, a combined approach of electrospinning a central vessel and casting a hydrogel for self-assembly of ECs was investigated to generate a perfusable EC-network.

Methods

To produce a perfusable and porous tube polycaprolactone (PCL) was used in an electrospinning process. After electrospinning the tube was treated with sodium hydroxide to increase the hydrophilicity. A hydrogel containing rat tail collagen, human umbilical vein endothelial cells expressing green fluorescent protein (GFP-HUVEC), and human adipose tissue derived stromal cells (hASC) was cast directly onto the PCL tube. The tube was cultivated in a custom-made bioreactor either under perfusion or static conditions. Extracellular matrix and cellular organization was analyzed via GFP fluorescence during the cultivation period and via immunohistochemistry for collagen I and collagen IV after the end of the experiment.

Results

Casting a hydrogel onto the PCL electrospun tube resulted in a homogeneous distribution of collagen in between the nanofibrous structure, which was demonstrated by a staining for collagen I. The development of an EC network was detected during cultivation under perfusion. In cryosections hollow structures in the EC network were not only present as a layer covering the tube but could also be detected in the wall of the PCL tube. Through a collagen IV staining the basement membrane surrounding the EC was detected.

Conclusions

The infiltration of a PCL-tube with a cell-containing hydrogel leads to EC network formation with hollow structures in the PCL-tube wall. The EC network formation in the PCL-tube can be used to receive sprouting form the PCL-tube into tissue or to connect the PCL-tube with a vascularized hydrogel. This represents a first promising and mandatory step towards the establishment of a fully perfusable tissue engineered constructs.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_699 Naturally-derived bioactive phytoprotein promotes bone defect repairs through neuro-osteogenic crosstalk. Augustine S. Brah[1 2], John A. Kubi[1 2], Kenneth M.C. Cheung[1 2], Kelvin Yeung[1 2]

[1]Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong, Hong Kong; *[2]Shenzhen Key Laboratory For Innovative Technology in Orthopaedic Trauma, HKU-Shenzhen Hospital, Shenzhen, China*

Introduction/Objectives

The increase in delayed bone defect healing remains a global challenge to orthopaedic surgeons despite improvements in treatment modalities for bone fractures. Neuro-osteogenic crosstalk has recently become a pivotal bone defect repair target. The neuro-osteogenic crosstalk mediated by neurotrophic factors, netrins, and neuropeptides modulates osteoblast activities to promote bone regeneration. Appropriate neuro-osteogenic stimulation could therefore influence bone remodeling process during bone defect healing. Thus, the development and application of bioactive agents that can stimulate optimal neuro-osteogenic cues to facilitate bone regeneration is essential for improving bone defect repairs. In this study, we investigated the neuro-osteogenic potential of *Dioscorea opposita* Thunb-derived 'HKUOT-S2' protein in improving bone defect repairs. We have provided intriguing findings from this newly isolated low-molecular-weight osteogenic "HKUOT-S2" protein (32kDa) which possesses neuro-osteogenic properties for faster bone defect repairs.

Methods

A 0.9 mm circular femoral bone defect was created in 8-week-old C57BL/6N mice. The HKUOT-S2 protein (2.18 mg/Kg) was administered by direct subcutaneous (s.c) injection at the defect site three times per week for 4 weeks post-surgery. Micro-computed tomography (μ-CT) scan was taken weekly to assess the HKUOT-S2 protein-induced bone defect repairs. In addition, retinoic acid-induced neuro2a cell differentiation with or without (w/o) HKUOT-S2 protein treatment was performed *in vitro*, and the condition media (CM) was collected for downstream applications to assess the neuro-osteogenic functions of HKUOT-S2 protein. The neuronal differentiation CM w/o the HKUOT-S2 protein treatment was used to differentiate 8-week-old C57BL/6N mouse-derived primary bone marrow mesenchymal stem cells (BMSCs) into osteoblasts. A Student's t-test was used for all statistical analyses using GraphPad Prism 8.0 (GraphPad Software, CA, USA). The values were shown as mean ± SEM. *$p < 0.05$ was considered significant.

Results

The HKUOT-S2 protein treatment significantly enhanced bone defect healing within 4 weeks by increasing bone mineral density (BMD), Bone volume fraction (BV/TV), and Trabecular thickness (Tb.th) compared to the sham control group which received only 1x phosphate-buffered saline (1X PBS). The HKUOT-S2 treatment also promoted periosteal sensory nerve activities by increasing the expression of neuronal markers CGRP, MAP2, GAP43, and PGP9.5 at the bone defect site. the HKUOT-S2-induced neuromodulatory activities corresponded with increased osteogenic activities through upregulation in OPN and BMP2 expressions at the bone defect sites. The in vitro results also showed that HKUOT-S2 protein treatment promoted neuro2a (N2A) cell differentiation by increasing neurite extension and enhanced expression of neuron differentiation markers GAP43 and PGP9.5. The HKUOT-S2-induced N2A cell differentiation condition media (CM) also improved BMSCs-derived osteoblasts differentiation and biomineralization.

Conclusions

The HKUOT-S2 protein can serve as a promising neuro-osteogenic crosstalk modulator for accelerating bone regeneration and defect healing.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_808 A new ovine osteomyelitis model for the evaluation of intramedullary implants with bactericidal surfaces. sacha cavelier[1 2 3], Siamak Saifzadeh[4 5], Ronja Finze[3 6], Alba Cisneros Martin[1 3], Olivia Richardson[1 3], Silvia Cometta[1 3 7], Flavia Medeiros Savi[1 3 4], Dietmar Hutmacher[1 3 7]

[1]Max Planck Queensland Centre for the Materials Science of Extracellular Matrices, Queensland University of Technology, Kelvin Grove, Australia; *[2]ARC Training Centre for Cell and Tissue Engineering Technologies, Queensland University of Technology, Kelvin Grove, Australia*; *[3]School of Mechanical, Medical and Process Engineering, Queensland University of Technology, Kelvin Grove, Australia*; *[4]Centre for Biomedical Technologies, Queensland University of Technology, Kelvin Grove, Australia*; *[5]Medical Engineering Research Facility, Queensland University of Technology, Chermside, Australia*; *[6]Department of Hand, Plastic and Reconstructive Surgery, BG Trauma Center Ludwigshafen, University of Heidelberg, Heidelberg, Germany*; *[7]ARC Training Centre for Multiscale 3D Imaging, Modelling, and Manufacturing, Queensland University of Technology, Kelvin Grove, Australia*

Introduction/Objectives

Fractures of the tibial shaft represent an incidence of 17–21 per 100,000 population and approximately 71% are treated with tibial nail implantation. One of the main postoperative complications is surgical site infection and osteomyelitis (infection of the bone tissue), which occurs in 14.9% of open fractures. Treatments are challenging, as they usually include the surgical removal of the infected tissue and prosthesis and a multi-drug, high-dose antibiotic medication plan with the associated complications. One approach to combat this clinical problem is the development of titanium nails with bactericidal surfaces, such as metallic ions coatings. Despite its intensive use throughout recorded history for its sanitizing properties, copper has not yet been approved to be incorporated in orthopaedic or trauma implants for use in clinical settings. Copper-coated titanium implants must be evaluated *in vivo* before regulatory processes and commercialization. The large animal osteomyelitis models reported in the literature are primarily used for antibiotics-coated surfaces and are unadapted to the specificities of copper-coated surfaces. This study aims to establish a new osteomyelitis model designed to assess copper-coated intramedullary implant's efficacy.

Methods

The proposed model is a reproducible ovine early-stage model of tibial infection using *S. aureus* as a bacterial inoculum. Control and experimental groups (n=4) received, respectively, a sterile saline and a low

dose of bacteria solution (10^4 Colony-Forming-Unit, or CFU). After insertion in the tibial intramedullary canal of a shorter, customized titanium non-coated nail, the inoculum was injected with a spinal needle. This active inoculation method was selected to reflects the clinical scenarios where 73% of contaminations in open fractures occur pre- and intra-operatively by penetrating the trauma itself. Sheep underwent daily temperature monitoring, and blood culture and blood analysis every two days during the experimental period of seven days. Implant, bone tissue, bone marrow and synovial joint liquid samples were collected for CFU counting on *S. aureus*-selective and non-selective agar plates.

Results

Blood culture and CFU count in the control group samples were negative, demonstrating the absence of cross-contamination. Bacteriological analyses in the experimental group samples indicated that *S. aureus* growth was detected on the implant surface (10^5 CFU/mm^2) and surrounding tissues, primarily in the proximal region (up to 10^6 CFU/mm^2 concentration) supposedly due to the obstruction of the diaphyseal region by the implant. Blood culture analyses were negative, revealing the absence of concomitant systemic infection.

Conclusions

By concentrating the bacteria at the implant's surface and in the proximal region of the tibial canal in seven days, this infection model lays the foundations for the future evaluation of copper-coated surfaces that are efficient against bacteria via contact-killing mechanisms over a short period. The future *in vivo* testing of the efficacy of bactericidal copper-coated implants in large animals will represent a step forward in the commercialization of such implants.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_813 3D Composite Cone Scaffold Development: Determination of Structural, Functional and Antibacterial Properties. Arden Çınar[1 2], Gözde Köle[3], Nihan Ünübol[4 5], Halime Kenar[6 7 8]

[1]Biomaterials Center, Acibadem Mehmet Ali Aydinlar University, İstanbul, Türkiye; *[2]Department of Biomedical Engineering, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, İstanbul, Türkiye*; *[3]Biomaterials Center, Acibadem Mehmet Ali Aydinlar University (ACU), Turkiye, İstanbul, Türkiye*; *[4]School Of Medicine, Department Of Basic Sciences, Medical Microbiology, Acibadem Mehmet Ali Aydinlar University, İstanbul, Türkiye*; *[5]Medical Laboratory Technician Program, Vocational School of Health Services, Acibadem Mehmet Ali Aydinlar University, İstanbul, Türkiye*; *[6]Biomaterials Center, Acibadem Mehmet Ali Aydinlar University (ACU), Turkiye, Department of Biomedical Engineering, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Turkiye, Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Turkiye, İstanbul, Türkiye*; *[7]Department of Biomedical Engineering, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Turkiye, İstanbul, Türkiye*; *[8]Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Turkiye, İstanbul, Türkiye*

Introduction/Objectives

This study aims to design and produce polymer-based composite bioactive scaffolds with improved mechanical and antibacterial properties for bone regeneration. The approach involves synthesizing mineral-tannic acid (M-TA) microparticles, incorporating them into poly(lactic acid) (PLA) to form composite filaments, and using these filaments to create 3D-printed scaffolds through fused deposition modeling.

Methods

Mineral-tannic acid microparticles were synthesized by adding tannic acid to simulated body fluid, and their morphology and composition were validated through scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR), respectively. These microparticles were then incorporated into PLA filaments through extrusion, and scaffolds with defined structural characteristics were produced using 3D printing. Collagen was adsorbed on the scaffolds and crosslinked with tannic acid to enhance cell adhesion. SEM analysis was performed to measure scaffold pore size and filament thickness, and compression test was performed to determine their mechanical strength. For cytocompatibility assessment, human fetal osteoblast (HOb) cells were seeded on PLA and PLA/M-TA/Col scaffolds, stained with FITC-Phalloidin, and imaged with confocal microscopy. The Presto Blue assay was used to measure cell proliferation, and osteogenic differentiation was evaluated by bone mineral deposition. The antibacterial properties of the microparticles against *S. aureus* were studied with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, and scaffolds' antibacterial property was also evaluated.

Results

SEM revealed that M-TA microparticles had an average size of 1.08 ± 0.18 μm. The PLA and PLA/M-TA scaffolds showed dimensional accuracy, with strip widths of 587 ± 48 μm for PLA and 634 ± 26 μm for PLA/M-TA, and pore sizes of 455 ± 7 μm and 433 ± 17 μm, respectively. Mechanical testing showed that PLA/M-TA scaffolds exhibited significantly higher, almost 2 times higher, Young's Modulus than PLA scaffolds. Confocal microscopy revealed better cell adhesion and spreading on the collagen-coated PLA/M-TA scaffolds. In terms of cell growth, PLA scaffolds led to the highest proliferation rate, followed by PLA/M-TA and PLA/M-TA/Col scaffolds. Osteogenic differentiation was most pronounced on PLA/M-TA/Col scaffolds, which had the highest bone mineral deposition, followed by PLA/M-TA and PLA scaffolds. The tannic acid and M-TA microparticles showed a bactericidal activity against *S. aureus,* while the scaffolds containing the microparticles reduced the microbial load in a concentration dependent manner.

Conclusions

The M-TA microparticles were successfully integrated into the 3D-printed PLA scaffolds with an even distribution. M-TA microparticle incorporation enhanced PLA scaffold's biomimicry, and mechanical performance, and provided antibacterial property, while collagen coating further improved cytocompatibility and osteogenic differentiation. These results contribute to advancing bone tissue engineering and offer a promising direction for future research and application in treatment of bone defects.

Topic: Submit to SYMPOSIUM

Subtopic: The role of osteoblasts/osteoclasts in bone cell interaction models in vitro - tools for 3Rs-compliant analysis of drugs and biomaterials in the bone context.

Type: Poster presentation

TERMIS25_856 A new in vitro (humanized) bioassay mimicking osteoclast bone remodeling.

Mick Gagliardi[1], Alexander Sieberath[2], Daniël Molin[1], Jochen Salber[2], Nynke van den Akker[1]

[1]*Maastricht University, Maastricht, Netherlands*; [2]*Ruhr-Universität Bochum, Bochum, Germany*

Introduction/Objectives

This study aimed to establish an osteoclast functionality bioassay that will allow us: 1) to monitor osteoclast (OC) vs osteoblast (OB)-homeostasis, and 2) to validate bone disorder therapies, i.e. in vitro testing the efficacy of drugs and materials on bone homeostasis. This bioassay is built by using optimized co-cultures of murine MC3T3-E1-derived OBs and RAW264.7-derived OCs, cultured on Calcium-Phosphate coatings (CaP) and on the patented in vitro bone matrix analogue Osteomimic (OM). Next, we aim to fully humanize this assay, making use of human cell lines, to enable clinical translation (versus murine cells) and reproducibility (versus primary cells).

Methods

MC3T3-E1 and RAW264.7 cell lines were cultured on their respective OB/OC differentiation regimes, on either tissue culture plastic (TCP), CaP or OM coating. Also, cells differentiated on TCP and subsequently detached, were transferred to CaP/OM coating. After co-incubation of these differentiated OB/OC cells on CaP/OM coating, Calcein was added to visualize and analyze degradation and generation of extracellular (bone) matrix, next to cell-specific markers phalloidin and Hoechst. For this, fluorescent high content imaging was applied and combined with image cytometry to qualify and quantify the efficiency of co-culture, in vitro matrix metabolism and remodeling. Furthermore, optimization of OC-differentiation of human monocytic cells (THP-1 cell line) was executed. To determine OC differentiation, mRNA expression profiling and TRAP-staining was performed.

Results

The developed bioassay, making use of a murine OB/OC co-culture system on CaP and OM coating, proved to be a solid model for modeling and analyzing bone matrix remodeling. In this bioassay, MC3T3-E1 cells were differentiated towards OB on the coated plates, whilst RAW264.7-cells were differentiated towards OCs on TCP, viably detached and co-seeded on the OB-containing coated plates. Quantification and qualification of OB/OC bone matrix remodeling was executed effectively in mid- to high throughput using fluorescent imaging of Calcein. Differentiation of human monocytic THP-1 cells towards OCs revealed to be challenging, although mRNA expression profiling showed robust and promising data, with dynamic levels of the early OC-marker CSFR1, and steady increase of OC-markers MITF and SIGLEC15 over the course of a 7-day stimulation with either RANKL or RANKL + M-CSF following a PMA-induces macrophage differentiation step. TRAP-staining confirmed this differentiation, although polynuclearity so far remained limited to small numbers of max 3-4 nuclei per cell.

Conclusions

We built an up-and-running robust, reproducible and in vitro murine cell-line based functionality assay (bioassay) that can be used as a test system to validate the capacity/impact of drugs and materials on bone matrix remodeling. This model is currently being converted to a fully humanized bioassay, with data already showing promising results on the OC side. For the OB-part of the assay, the validated hFOB-cell line will be incorporated.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_944 The influence of actin severing and capping protein scinderin (adseverin) on early chondrogenesis. Tess Uphof[1], Andrea Lolli[1], Ruud Das[2], Eric Farrell[1]

[1]*Erasmus Medical Center, Rotterdam, Netherlands*; [2]*Scinus Cell Expansion Netherlands B.V., Zeist, Netherlands*

Introduction/Objectives

Mesenchymal stromal cell (MSC)-mediated bone tissue engineering via endochondral ossification represents a promising approach to the treatment of large bone defects. However, long culture periods impede translation of this approach to the clinic. In this study, we aimed to identify potential targets to accelerate the chondrogenic differentiation of MSCs. We determined that in our hands, a minimal differentiation period of 7 days is necessary for subsequent significant and reproducible bone formation *in vivo*. Bulk RNA sequencing was performed, comparing cells after 7 days of differentiation to cells after 3 days of differentiation, where little to no bone was formed. We focused on genes involved in cytoskeletal arrangement, a critical process during early differentiation. We identified scinderin (also known as adseverin), an actin severing and capping protein, as a differentially expressed gene. We aimed to elucidate

the role of scinderin during early chondrogenesis and target it in order to induce rapid chondrogenic differentiation.

Methods

Human paediatric MSCs were expanded and subsequently differentiated as cell pellets (200.000 cells) in differentiation medium (DMEM-HG+Glutamax, 50µg/mL gentamycin, 1.5µg/mL fungizone, 1% ITS, 40 ug/mL proline, 1mM sodium pyruvate, 0.1mM ascorbic acid, 10ng/mL transforming growth factor b3, 100nM dexamethasone). Bulk RNA sequencing was performed on pellets after 3 or 7 days of differentiation. The expression of scinderin was followed over time by RT-qPCR to reveal an expression pattern during chondrogenesis (N=4). In order to assess the role of scinderin during differentiation, a transient knockdown was performed on MSCs in monolayer, using transfection reagent lipofectamine and siRNA (20 nM). 2 days after transfection, chondrogenic differentiation was induced (N=4). Chondrogenesis was followed over time and assessed by RT-qPCR (COL2A1, COL10A1) and histology (GAGs).

Results

In the bulk RNA sequencing analysis, SCIN was identified as one of the most differentially expressed genes involved in cytoskeletal arrangement on day 7 compared to day 3 of chondrogenic differentiation. During chondrogenesis, the expression of scinderin was downregulated within the first day of pellet formation, after which the expression is upregulated, exceeding the monolayer baseline, until day 7. Since the expression of this protein naturally goes up during early chondrogenesis, we hypothesised that by knocking it down, the differentiation would be hampered. Surprisingly, the knockdown gave rise to an upregulation of COL2A1 and COL10A1 on day 7 compared to the scramble control. In histological sections at the same time-points, more GAG-rich matrix was formed in transfected pellets compared to scramble and untransfected controls. By day 14, this difference was diminished in both gene expression and histology.

Conclusions

Scinderin is an actin severing and capping protein that follows a distinct expression profile during chondrogenesis, alluding to a functional role in this process. Strikingly, even though this protein is naturally upregulated during early chondrogenesis, when knocking it down in monolayer prior to chondrogenic differentiation, a pro-chondrogenic effect was observed on day 7. By moduling scinderin’s expression, the early stages of chondrogenesis can be accelerated and will help to translate bone tissue engineering by making chondrogenic differentiation of MSCs faster and cheaper.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_1172 Evaluating demineralized bone matrix (DBM) remineralization: an ex vivo model for clinical assessment. Volker Eras[1], René Verboket[2], Daniel Kaltenhäuser[1], Jan Brune[1]

[1]German Institute for Cell and Tissue Replacement (DIZG, non-profit GmbH), Berlin, Germany; *[2]Goethe University Frankfurt, Department of Trauma Surgery, University Hospital, Frankfurt, Germany*

Introduction/Objectives

Demineralized bone matrix (DBM), pioneered by Urist and colleagues in the 1960s was developed to expose osteoinductive growth factors and promote bone healing[1]. While widely used clinically, high-quality data on DBM efficacy remains limited and context-dependent. This study explores the relationship between DBM remineralization and CT scan signal intensity. The primary aim is to investigate the correlation between cortical granulate calcium content and mean grey values (MGVs) in CT scans. Establishing this relationship could provide surgeons with a valuable tool for assessing DBM remineralization clinically.

Methods

Sample Preparation

Cortical granules (10 cm^3, 0.212-0.850 mm) from a single donor underwent demineralization for varying durations: 0 s, 5 s, 15 s, 30 s, 2 min, 5 min, 15 min, and 24 h (n=3 per timepoint).

Calcium Quantification

Complexometric titration was used to determine the calcium content of demineralized samples.

CT Imaging and Analysis

Cortical granules with varying mineral contents were used to fill a diaphysis segmentally before CT scanning. Mean grey values were measured using regions of interest (ROI) and correlated with the respective group's mineral content.

Results

Demineralization kinetics followed an exponential decay model. After 15 minutes, calcium content decreased to 22.8 ± 0.1% from the initial 24.1 ± 0.2%. CT scans showed increasing signal intensity corresponding to higher calcium content. Statistical analysis revealed a significant Pearson correlation between MGVs and bone granule mineral content ($p=0.009$; $R^2=0.92$).

Conclusions

This study demonstrates a strong correlation between bone allograft mineral content and CT scan signal intensity. These findings provide a foundation for future research on remineralization during bone remodelling. The ability to quantify different stages of remineralization opens new avenues for bone biology and regenerative medicine research.

1. Urist MR. Bone: formation by autoinduction. Science. 1965;150(3698):893-9.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_1348 Development of an injectable, photocurable, solvent-free emulsion-based scaffold for bone tissue engineering. Ece Özmen[1], Merve Unal[1], Serkan Dikici[1], Funda Tıhmınlıoğlu[1], Betül Aldemir Dikici[1]

[1]*İzmir Institute of Technology, İzmir, Türkiye*

Introduction/Objectives

Injectable and *in situ* polymerisable scaffolds are important for addressing irregularly shaped defects in hard-to-reach areas. These scaffolds enable minimally invasive application, maximum contact with the tissue while eliminating pre-design steps required in 3D printing. Bioactive agents and/or cells can be included in the composition of injectable scaffolds, allowing controlled release post-injection. Emulsion templating is a tunable technique that enables the production of porous matrices using water as porogen. This advantage enables high internal phase emulsions (HIPEs) to be used as injectable scaffolds if the other cytotoxic components can also be eliminated. This study aims to develop a solvent-free, polycaprolactone methacrylate (PCLMA) based HIPE composition as an injectable, *in situ* photocurable, porous synthetic scaffold for bone tissue engineering.

Methods

Pre-polymers with varying molecular weights and degrees of branching were synthesized, their structures and molecular weights were verified by spectroscopic and spectrometric methods. Viscosity, gel fraction, mechanical, accelerated degradation, and contact angle analyses were performed to compare the characteristics of the pre-polymers. Then, HIPE composition was developed by optimising the type/concentration of the photoinitiator and surfactant. After obtanining an injectable HIPE, morphological, mechanical, and *in vitro* cytotoxicity analyses were conducted, and *in vitro* biological characterisations are currently ongoing with human mesenchymal stem cells. The bone regeneration capacity of the scaffold will then be evaluated using an *ex vivo* chick femur defect model. 2-deoxy-D-ribose (2dDR) was introduced into

the emulsion composition as a pro-angiogenic agent, and its release was observed *via* Bial's assay. Following the dose optimisation, the pro-angiogenic potential of the scaffold will be investigated using an *ex ovo* chick chorioallantoic membrane assay.

Results

PCLMA-based pre-polymers with a different number of arms and degree of polymerisation were successfully synthesised with at least 95% degree of methacrylation. Viscosity tests showed that a decrease in the molecular weight and the degree of branching decreases the pre-polymer viscosity for tested compositions. Mechanical and *in vitro* degradation analyses proved that a tightly packed network structure provides a higher degree of stiffness and causes slower degradation. Following the characterisation of pre-polymers, the solvent-free HIPE composition was successfully developed using the pre-polymer with the lowest viscosity. Injectability tests demonstrated that the shear-thinning behaviour of the composition enables injection without clogging, while scanning electron microscopy showed the preservation of the porosity after injection. The cytocompatibility of the scaffolds was successfully confirmed, and further biological characterisations are currently underway. 2dDR was successfully incorporated into the emulsion composition without any phase separation, and its release profile was demonstrated.

Conclusions

This study demonstrates that HIPEs can be a highly promising alternative as injectable scaffolds for bone tissue engineering with the elimination of toxic components from the composition. Injectable PCLMA-based scaffolds show great promise as bone grafts and further studies will reveal their potential for clinical translation.

Topic: Tissue-Specific Focus

Subtopic: Bone

Type: Poster presentation

TERMIS25_1416 3D printed silk fibroin-alginate scaffolds for bone tissue regeneration. Rebekah Kay[1], Annalisa Tirella[1 2], Olga Tsigkou[1], Kaye Williams[1]

[1]*The University of Manchester, Manchester, United Kingdom*; [2]*The University of Trento, Trento, Italy*

Introduction/Objectives

Silk fibroin (SF), extracted from bombyx mori cocoons, is an excellent material for the development of in vitro bone scaffolds. SF is capable of supporting bMSCs growth, proliferation, and differentiation due to its biochemical signalling. However, SF bioinks have proven challenging for 3D bioprinting (3DBP) due to the production of beta sheets when the material is sheared, with uncontrolled impact on the mechanical properties. Alginate (alg) is a shear thinning material and an ideal bioink, but it does not provide sufficient signalling for cell adhesion. Here we report on the combination of SF, alg and oxidised alginate (OA) as bio inks for the 3DBP of bone mimicking scaffolds. Nano-hydroxyapatite (nHyA) was also included as a bioactive component and used at w/v fractions suitable with 3DBP.

Methods

Due to the low viscosity of the bioink produced, a technique known as suspended layer additive manufacturing (SLAM) was used: an agarose fluid bath, a 20G nozzle, 50μL/min flow rate, and 3mm/s speed, were set to 3DBP scaffolds with known macroporosity. The mechanical properties of the hydrogels were controlled via ionic cross-linking with $CaCl_2$, whereas the microporosity was controlled by an optimised freeze-drying step (i.e., freezing steps prior to lyophilisation). The scaffolds were characterised in stability, pore size distribution, and mechanical properties up to 6 weeks. A preliminary biological study with bMSCs was carried out to assess the optimal concentrations of polymers and nHyA.

Results

The resolution of the 3DBP was found to be 1.6mm with a line height of 0.25mm, which was maintained with following lyophilisation steps. 3DBP scaffolds supported bMSCs growth, with clear increases in metabolic activity over time (up to 4 weeks). It was found that while scaffolds containing either SF or gelatin had equivalent metabolic activities at each time point, those with SF actively promoted better adhesion of bMSCs, exhibiting cell morphology typical of osteoblasts. An increase in adhesion and growth of the bMSCs was observed with the inclusion of nHyA and this was also increased with higher content.

Conclusions

The proposed manufacturing method enable fabrication of scaffold with a engineered hierarchical structure and controlled mechanical properties for 3D in vitro bone tissue models. SF has been successfully combined with alg, OA, and nHyA to 3DBP in vitro scaffolds with controlled pore size at the macro- and micro-level typical of bone tissue. Homogeneous dispersion of nHyA was achieved, and the scaffolds were found to be stable in cell-culture conditions for up to 6 weeks. Scaffolds with SF show promising results in supporting bMSCs growth, with morphology typical of osteoblasts, and the inclusion of nHyA has been seen to actively promote the adhesion and growth rates.

Topic: Submit to SYMPOSIUM

Subtopic: Sustainable biomaterials in biofabrication and tissue engineering

Type: Poster presentation

TERMIS25_301 Novel ionically conductive biomaterial scaffolds for cardiovascular tissue engineering. Aleksandra Serafin[1], Maurice Collins[1]

[1]*University of Limerick, Limerick, Ireland*

Introduction/Objectives

Cardiovascular diseases (CVD) are the leading cause of death in the world, as well as a major contributor to healthcare costs and economic losses. To combat this, a new trend in the field of tissue engineering (TE) has emerged in the form of conductive biomaterials. It has been known that cells are affected by electrical stimulation, especially cells of a conductive nature such as heart cells. The application of conductive TE scaffolds to CVD models aims at repairing and improving the growth and signalling of damaged myocyte by facilitating electrical stimulation. The major drawbacks of using standard conductive additives such as PEDOT:PSS or carbon nanofibers arise from low conductivity in neutral pH, insolubility in water and poor biocompatibility. In addition, the complete degradation of these electroconductive scaffolds after the therapeutic treatment is completed is not fully understood, with the renal clearance of these additives often being questioned. The focus of this research project centres on raising the conductivity of the scaffolds in an ionic manner, by incorporating sodium and potassium ions, which more closely replicates the natural nerve signalling of an action potential expressed to contract the heart and enable blood flow around the body, which has not been explored to date. Further incorporation of the ions with bioactive sustainable components such as gelatin, elastin and hyaluronic acid allow for the development of novel conductive biomaterials.

Methods

Techniques used to characterise these novel conductive biomaterials include compression, FTIR, swelling studies, rheology, and in-vitro biocompatibility studies.

Structural analysis: The morphology of the developed hydrogels was analyzed using SEM. Cross-sectional images of hydrogels were analyzed to evaluate the changes in the morphology and porosity.

Compression Properties: The compression test was carried out using an in-house compression test facility equipped with a 1 kN load cell, with a screw speed of 1 mm/min up to 60% strain. The Young's Modulus of the samples was calculated as the slope in the linear region of a normalized stress vs. strain graph.

Conductivity Properties: The resistivity of the hydrogels was measured using an Ohm meter. The conductivity was then calculated as follows:

$\sigma = lRA$

Where, σ is the conductivity, l is the sample length, A is the cross-sectional area, and R is the resistance.

Results

The developed ionically conductive hydrogels display adequate mechanical strength comparable to that of the native tissue. The incorporation of sodium and chloride ions at different concentrations ranging from 1-3M increased the conductivity of the samples to varying degree. The incorporation of 3M KCl ions into the hydrogel raised the ionic conductivity of the samples most favourably to $6.06x10^{-5}$ S/cm, especially when compared to the 3M NaCl samples.

Conclusions

From this work, it can be concluded that the developed ionic conductive hydrogels show promise as a TE strategy for CVD repair.

Topic: Submit to SYMPOSIUM

Subtopic: Innovations in Cardiovascular Tissue Engineering: Paving the Way for Next-Generation Medical Devices

Type: Poster presentation

TERMIS25_319 Anisotropic surface topography improves the behaviour of venous endothelial cells (VECs) from cardiovascular patients. Christien Beez[1 2 3], Miriam Ermer[4], Vasileios Exarchos[2 3], Heike Meyborg[1 2 3], Katrin Krüger[2 3], Volkmar Falk[2 5 6], Maximilian Emmert[2 6 7], Timo Nazari-Shafti[1 2 6]

[1]Berlin Institute of Health at Charité–Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), Berlin, Germany; *[2]Deutsches Herzzentrum der Charité (DHZC), Department of Cardiothoracic and Vascular Surgery, Berlin, Germany*; *[3]Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany*; *[4]Deutsches Herzzentrum der Charité, Department of Cardiothoracic and Vascular Surgery, Berlin, Germany*; *[5]Department of Health Sciences and Technology, ETH Zurich, Zürich, Switzerland*; *[6]German Centre for Cardiovascular Research (DZHK), Partner Site Berlin, Berlin, Germany*; *[7]Institute for Regenerative Medicine, University of Zurich, Zürich, Switzerland*

Introduction/Objectives

Cardiovascular implants (CVIs) have revolutionized the treatment of patients with cardiovascular diseases (CVDs), yet thrombogenicity remains a major challenge for their safe and effective use. One promising strategy to reduce and even avoid the unwanted formation of blood clots is the improvement of implant endothelization through surface modification.

This present study investigates the *in vitro* effect of two distinct surface topographies - flat and anisotropic micro-grids structure - on venous endothelial cells (VECs) isolated from CVD patients. Given the medical history of each patient, this study additionally explores whether the number of CVD risk factors or the presence of *diabetes mellitus* influence VECs response to both topographies.

Methods

VECs were isolated from the great saphenous vein of eight patients and cultivated for up to eight passages. Cell properties, including morphology, proliferation, senescence, and angiogenesis, were analysed after up to five days of culture on glutaraldehyde cross-linked gelatin-coated silicone surfaces with either a flat or micro-grid structure.

Results

Our findings indicate that the anisotropic micro-grid surface positively altered VEC characteristics, since cells cultured on this surface displayed a significantly increased aspect ratio, cell-cell connectivity, and

proliferation as determined by fluorescence microscopy of vascular endothelial cadherin (VEC) or KI-67, respectively. These beneficial effects occurred regardless of the number of patient risk factors or the presence of *diabetes mellitus*. Furthermore, VECs grown on micro-grids exhibited a marked reduction of the frequency of senescence-associated-β-galactosidase positive cells. This sign of reduced cellular ageing appears to be independent of risk factors or *diabetes mellitus* as comorbidity. One prominent observation of this study was the enhanced angiogenic potential of VECs when cultured on micro-grids. In tube formation assays, the number of junctions as well as the total branching length was significantly enhanced for VECs that have been cultured prior to the assay on micro-grids in comparison to VECs cultured on flat surfaces. This enhanced angiogenic capacity was confirmed in wound healing assays, where VECs on micro-grid surfaces aligned orthogonally to the wound edge achieved faster closure than compared to both parallel micro-grid orientation and flat surfaces. Notably, these effects were consistent across patients and unaffected by individual risk profiles or diabetes status.

Conclusions

In summary, our results emphasize that cultivation on anisotropic micro-grids yielded noteworthy improvements in proliferation, cell junction integrity, reduced senescence, and angiogenesis of endothelial cells from CVD patients. It vastly suggests that this surface may be beneficial for endothelization of CVIs, which underscores the general potential of modified surfaces to enhance the performance of implants.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_349 A hierarchically structured biohybrid vascular graft for small diameter blood vessels: Nanofibrous-microporous design with enhanced mechanical and cytocompatibility properties. Gabriela Morais da Costa[1], Janaina de Andréa Dernowsek[2 3], Thais Amaral França[2], Pamela Ferreira Do Nascimento[2], Claudia Larissa Viana Da Silva[2], Sônia Maria Malmonge[1]

[1]Postgraduate Program in Biomedical Engineering, Federal University of ABC, São Bernardo do Campo, Brazil; *[2]Quantis Biotechnology, São Paulo, Brazil*; *[3]3D Biofabrication Training and Innovation Center, BioEdTech, São Paulo, Brazil*

Introduction/Objectives

Synthetic vascular grafts for small-caliber arteries (<6 mm) typically have low patency rates and suffer from neointimal hyperplasia, mainly due to their limited ability to support a functional endothelium. To overcome these challenges, we present a promising strategy to recapitulate native artery' structural heterogenicity, wherein a triple-layer hybrid vascular scaffold (HVS) with radially oriented fibers and porous interconnected network is designed to provide adequate mechanical support and biocompatibility combining synthetic and natural bioresorbable polymers via solution blow spinning (SBS) and 3D printing techniques.

Methods

The HVS was fabricated by SBS with 5% poly(ε-caprolactone) (PCL) in chloroform and 3D printed hydrogel made of 1% polyethylene glycol (PEG), 3% gelatin, and 3% alginate (PAG). A rotating collector was employed as a receiving platform for SBS process to create the inner layer of HVS. Subsequently, 3D printed hydrogel filaments were deposited, and then coated with spun PCL nanofibers. This assembly yielded a "sandwich-like" structure, characterized by its hierarchical nanofibrous and microporous architecture. The HVS was analyzed by scanning electron microscopy (SEM) for morphological characterization, contact angle measurement to evaluate wettability, and subjected to tensile stress-strain, suture retention and burst pressure tests. The rheological characterization of the hydrogel was performed with a flat parallel plate of 25 mm in diameter. Cell proliferation (Alamar Blue Assay) and cell adhesion via SEM assays were performed on days 1, 2 and 3 to evaluate the biological response of fibroblasts (L929).

Results

Tubular structures were successfully generated with an internal diameter of 3.8 mm and total wall thickness of 1.2 mm. The nanofibers presented a circumferentially oriented fibrous matrix with an average diameter of 243,64 ± 0,05 nm. Microporous structure was observed in the middle layer, promoting an interconnected network between the fibrous layers. The contact angle revealed that HVS has high hydrophilicity, with an initial contact angle of 41.30° ± 1.41°. The hydrogel presented pseudoplastic shear thinning behavior, with decreasing viscosity as the shear rate increased, indicating high printability and making it suitable for cell encapsulation. The adopted configuration improved the mechanical properties, with ultimate tensile strength (UTS) of 1.03 ± 0.14 MPa and Young's modulus (E) of 3.27 ± 0.30 MPa, within the range of natural blood vessels (UTS = 1-2 MPa; E = 0.26-14.5 MPa), in addition to suture retention (3.75 ± 0.54 N) and pressure burst (up to 3040 mmHg), higher than those of the human coronary artery. Regarding cytocompatibility, it was observed that fibroblasts adhered well to the surface and maintained metabolic activity of 90.16 ± 1.85% on the third day of culture.

Conclusions

Our findings indicate the potential of HVS fabricated by combined SBS and hydrogel 3D printing techniques as tissue engineered vascular grafts for small-diameter blood vessels. The favorable performance is due to high wettability, mechanical properties compatible with native vessels, and hierarchical nanofibrous/microporous architecture, providing an ideal substrate for cell adhesion and rapid remodeling of the scaffolds. The development of these scaffolds represents a relevant advance in the treatment of cardiovascular diseases, with feasibility for scalable production.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_605 Vascular remodeling: an in-vitro investigation of the role of mechanical vibrations. Elena Carrara[1], Sofia Poloni[2], Marta Ripamonti[1], Kristian Valen-Sendstad[3], Andrea Remuzzi[2], Michela Bozzetto[1], Chiara Emma Campiglio[2]

[1]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy; *[2]University of Bergamo, Dalmine, Italy*; *[3]Simula Research Laboratory, Oslo, Norway*

Introduction/Objectives

The mechanobiology of vascular cells plays a key role in vascular remodeling and evidence showed that turbulent hemodynamics influence vascular cells functions in vitro. Recent numerical simulations investigated the effect of mechanical stresses on vessels remodeling, and revealed that “turbulent-like” blood flow induces vascular wall vibrations. However, the biological basis of this phenomenon remains unclear. This preliminary study aims at investigating the effects of vibrations on human umbilical vein endothelial cells (HUVECs) and aortic smooth muscle cells (HASMCs) by exposing cells to mechanical vibrations and by assessing their impact on cell viability and localization of the transcription factor YAP (yes-associated protein 1) that correlates with athero-protection.

Methods

HUVECs and HASMCs were seeded in 6-well plates. For each cell type, one seeded plate was subjected to vertical vibrations (75, 150, and 550 Hz frequencies), while a second plate was maintained in static for 1h or 24 h. At the end of the experiment, cells viability was analyzed by LDH assay and vibrations effect on cell mechanotransduction was investigated by assessing YAP localization. An immunofluorescence staining was performed to assess any possible changes in cell morphology and cell adhesion to the substrate and neighboring cells.

Results

Vascular cells were subjected to vertical displacements at 75, 150, and 550 Hz frequencies. LDH assay revealed a significant increase in LDH release in HUVECs exposed to 150 Hz-vibrations for 1 h compared to controls, while no viability differences were observed at the same time in HASMCs. The analysis of YAP immunostaining in endothelial cells showed an increase in nuclear YAP at 24 hours with 150 Hz-vibrations, suggesting the nuclear translocation of the factor, potentially correlating with an athero-prone signaling. A preliminary analysis of VE-cadherin on HUVECs suggests a different aspect of cell-cell interactions comparing the vibration condition to the control one.

Conclusions

Our study demonstrates the device's applicability in exploring the effects of mechanical vibrations on HUVECs and HASMCs cultures. Preliminary results suggest exposure to 150 Hz-vibrations affects HUVECs viability early on, but the effect seems to diminish over time. Conversely, HASMCs are not sensitive to the stimulation in terms of viability. Our device will allow investigating cell functional changes involved in vascular remodeling and cell dysfunctions responsible for cardiovascular diseases.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_653 Development of novel hydrogel composites with dual anti-platlet and anti-bacterial functionalities. Dikshita Madkatte[1 2], Marcus Soter[1 2], Thi-Huong Nguyen[1 3]

[1]*Institut für Bioprozess- und Analysenmesstechnik e.V. (IBA), 37308, Heilbad Heiligenstadt, Germany*; [2]*Karlsruhe Institue of Technology, 76131, Karlsruhe, Germany*; [3]*Institute for Chemistry and Biotechnology, Faculty of Mathematics and Natural Sciences, Technische Universtät Ilmenau, Ilmenau, Germany*

Introduction/Objectives

Cardiovascular disorders are the leading cause of death globally, and thrombosis and bacterial contamination pose major challenges in cardiovascular tissue engineering. Synthetic implants often lack extracellular matrix (ECM) properties, increasing the risk of thrombosis and bacterial contamination. Hydrogels have been developed to mimic vascular ECM, supporting cell growth and tissue repair. However, they still face limitations in mechanical strength, degradation, and anti-fouling properties. Developing biocompatible and anti-bacterial biomaterials is crucial for progress in cardiovascular tissue engineering. We developed a novel ECM-inspired anti-fouling Photocrosslinkable hydrogel composites comprising gelatin methacrylate (GelMA), elastin methacrylate (ElMA), and glycol chitosan methacrylate (ChiMA) to create a biomaterial system that prevents both thrombosis and bacterial contamination.

Methods

Photocrosslinkable hydrogel composites (10% w/v) using GelMA, ElMA, and ChiMA were prepared by blending GelMA:ElMA in a 70:30 ratio and varying ChiMA content to mimic vascular ECM with anti-fouling properties. Biomaterial films were fabricated by spin coating and UV curing with lithium phenyl-2,4,6-trimethyl benzoyl (LAP) photoinitiator. Material characterization was performed by rheology, surface wettability, swellability, and zeta potential. The anti-fouling behavior was assessed through platelet and bacterial adhesion tests. Confocal laser scanning microscopy (CLSM) was used to image hydrogel composites, and platelet and bacterial attachment was quantified.

Results

Novel hydrogel composites of different blending ratios were successfully synthesized using GelMA, ElMA, and ChiMA. They demonstrate tunable mechanical properties with storage moduli from 100 Pa to 500 Pa, contact angle from 14° to 24°, swellability from 0% to 550%, and zeta potential between -1 mV and -25 mV. The potent anti-fouling abilities of hydrogel composites were observed by inhibition in platelet

and/or bacterial attachment up to 70%. The variations in platelet and bacterial responses observed on all the hydrogel composites are due to the differences in material characteristics.

Conclusions

An optimized ratio of 66.5:28.5:5 of GelMA:ElMA:ChiMA has been identified. This ratio has the potential to mimic vascular ECM for cardiovascular implant applications by preventing thrombosis and bacterial contamination, improving elasticity, and being a sustainable alternative to synthetic materials.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_703 Evaluation of Hb-functionalization and oxygen-binding capacity of hydrogel-based artificial erythrocytes. Tom Bode[1], Gesine Hentschel[1], Marc Müller[1], Birgit Glasmacher[1]

[1]*Institute for Multiphase Processes, Leibniz University Hannover, Garbsen, Germany*

Introduction/Objectives

The shortage of blood donations is a major issue in medical care, particularly for the perfusion of donor organs during transplantation. It is therefore necessary to develop alternatives to human blood transfusions to address this problem. In our previous study, human hemoglobin (Hb) functionalized artificial erythrocytes made from hydrogels were developed. The aim was to mimic red blood cells in terms of shape, size, rheological and mechanical properties, and gas transport capacity. The oxygen binding capacity showed a sharp decline over time. In this study, the artificial erythrocytes' functionalization protocol with human hemoglobin is therefore redesigned and the oxygen-binding functionality reassessed.

Methods

Poly-sodium acrylate-co-acrylamide (P(SA-Am)) hydrogel beads were prepared using a microfluidic system, fabricated via microwire molding technique. The beads were functionalized (static conditions, dark environment, 4°C) with Hb by incubating in an 8 mg/mL Hb solution for 4 h on a shaker plate, followed by cross-linking for 1.5 h with a 5% (v/v) glutaraldehyde solution. To further enhance functionalization, the beads were again incubated with 8 mg/mL Hb solution for 1 hour. After three cold wash steps with PBS, the beads were incubated in PBS overnight. Subsequent experiments were performed after washing the particles again with PBS. The composition of the surface coating was analyzed by FTIR spectroscopy. Oxygen binding was qualitatively assessed by chemiluminescence using a luminol assay.

Results

Functionalization with Hb caused a color change from clear to a reddish-brown hue. FTIR measurements of the Hb-functionalized beads, compared to non-functionalized beads and pure Hb, showed characteristic peaks of Hb in the measured spectra, particularly in the amide I, II and III bands (1654, 1541 and 1312 cm-1), indicating the presence of Hb on the bead surface. Hb functionalization resulted in chemiluminescence upon addition of luminol. 1 g of functionalized beads showed the same luminescence intensity (0.45% of the maximum sensor intensity) as 1 ml of the 8 mg/ml Hb solution. 0.125 g of beads show an intensity similar to 0.2 mL of 2 mg/mL Hb solution (0.089%). The previously used protocol resulted in a luminescence intensity of less than 0.049%.

Conclusions

The study shows that hydrogel beads have been successfully functionalized with human Hb, improving the protocol by increasing Hb concentration and cross-linking time and increasing their potential as artificial erythrocytes. The observed changes in color, chemiluminescence as well as the FTIR results indicate that the artificial erythrocytes contain Hb at their surface and within the particles' outer shell and have the capacity to bind oxygen to a higher degree than previous iterations. However, a quantitative evaluation of the oxygen-carrying capacity and release kinetics is still necessary. Further work will address long-term stability and

potential degradation under various physiological conditions as well as possible immunogenic responses and toxicity concerns especially in blood contact.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_790 Growing biohybrid scaffolds in the lab: PGS-M PolyHIPE Scaffolds for Vascular Tissue Engineering. Rosanna Hood[1]

[1]*University of Sheffield, Sheffield, United Kingdom*

Introduction/Objectives

Cardiovascular disease is the major cause of death globally, with over 17 million deaths worldwide. [1] Currently surgical procedures like coronary bypass surgery and percutaneous coronary interventions remain the gold standard, but in cases where no autologous vessel can be used, synthetic grafts are the alternative. Whilst successful at large diameters, this solution has poor patency for diameters less than 6 mm and fails to facilitate new tissue growth *in vivo*. [2] Tissue engineered vascular grafts (TEVG) are aimed to fill this demand. Biohybrid scaffolds, created through the culture of cells on synthetic scaffolds, combine the structural and mechanical properties of synthetic grafts and the biological components of native tissue, through the deposition of relevant extracellular matrix (ECM) and subsequent decellularization. This results in a biomimetic graft encourages cell adhesion and proliferation and has off-the-shelf availability for surgical implantation.

This project aims to optimise the previously developed method of poly(glycerol sebacate) methacrylate (PGS-M) polymerised high internal phase emulsions (polyHIPE) to produce highly porous and customisable grafts for vascular replacement. [3,4] By varying synthesis conditions and hence the structure of the polyHIPE scaffold, the effect of internal morphology on cell proliferation and ECM deposition will be investigated resulting in a fully optimised scaffold.

Methods

PGS and subsequently PGS-M have been synthesised in house via the two-step condensation reaction of equimolar amounts of sebacic acid and glycerol, followed by methacrylation to form PGS-M using methacrylic anhydride. Prior to UV curing, polyHIPEs of PGS-M were produced and the emulsion parameters were varied to create scaffolds with varying pore size distribution and interconnectivity. Human dermal fibroblasts were cultured for 28 days on the porous scaffolds to study infiltration and ECM deposition. Optimisation of the visualisation and quantification of the ECM produced has been completed using a variety of different histological stains and assays.

Results

PGS-M has been synthesised and used in the formation of porous polyHIPE scaffolds. The emulsion parameters were varied to create scaffolds with different internal morphologies and mechanical properties, which supported the long-term culture of immortalised HDFs. The metabolic activity, infiltration and collagen and elastin compositions were studied to highlight the most preferential scaffold for vascular graft engineering. Tubular grafts have been synthesised using the two most suited compositions to confirm this behaviour in the biologically relevant geometry.

Conclusions

Preliminary work in the creation of a biomimetic vascular graft has been completed and shows stability for long term cell culture with evidence of ECM deposition. Further research will be completed to highlight its promise as a replacement for autologous graft surgery. Investigation into decellularisation techniques, mechanical stimulation and culture conditions will be completed to increase ECM deposition and create a

biomimetic graft that encourages *in vivo* cell adhesion and proliferation whilst minimising the risks of intimal hyperplasia and thrombosis.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_997 Multicomponent Electroresponsive PLA-Based Scaffolds for Cardiac Tissue Regeneration: Abundant, Sustained and Prolonged Lactate Release. Pilar Adriana Haro Gutierrez[1], Samuele Colombi Montagna[2], Jordi Casanovas[3], Jordi Sans Mila[2], Elisabeth Engel Lopez[4], Hamidreza Enshaei[2], Jose Garcia Torres[5], Maria M. Pérez-Madrigal[2], Carlos Aleman[6]

[1]UNIVERSITAT POLITECNICA DE CATALUYA, BARCELONA, Spain; *[2]Universitat Politecnica de Cataluya, Barcelona, Spain*; *[3]Universitat de Lleida, Lleida, Spain*; *[4]Universitat Politecnica de Cataluya, Institute for Bioengineering of Catalonia, CIBER en Bioingeniería, Biomateriales y Nanomedicina, Barcelona, Spain*; *[5]Universitat Politecnica de Cataluya, CIBER en Bioingeniería, Biomateriales y Nanomedicina, Barcelona, Spain*; *[6]Universitat Politecnica de Cataluya, Institute for Bioengineering of Catalonia, Barcelona, Spain*

Recent studies have revealed that cardiac tissue regeneration is promoted by administering an initial dose of exogenous lactate and locally maintaining an abundant concentration of this compound for a prolonged period (*i.e.* around 10-14 days) through sustained release. The objective of this study was to design and develop a scaffold based on polylactic acid (PLA) that, combining different release mechanisms, achieves a sustained daily release of lactate, from the first day to the end of the recommended period.

The engineered electroresponsive scaffold was composed of three PLA microfibers (MFs) layers (1st, 3rd and 5th), which were prepared by electrospinning, and separated by spin coated lactate (2nd) and poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) (4th) intermediate layers. The hydrophobicity of the outer PLA layers (1st and 5th) allowed a delayed release of lactate from the intermediate 2nd layer over 3 days, which represents a remarkable improvement in terms of sustained release with respect to the almost immediate release (1-2 h) reported for lactate-loaded hydrogels. Furthermore, a complete lactate release was achieved when the 4th PEDOT:PSS layer was electrostimulated. Finally, the same scaffold was re-engineered to maintain the sustained release not only for a short period (3 days) but for a prolonged period (>10 days). For this purpose, the intermediate 3rd PLA layer was replaced by plasma-treated proteinase K-containing PLA MFs, which were produced by electrospun a PLA:enzyme mixture. The activity of the enzyme, which decomposes the ester bonds of PLA, combined with the effect of the plasma on the PLA structure, were found to produce a prolonged sustained release (>10 days) that, in addition, was modulated with the applied conditions. Thus, the daily average lactate release was of ∼3 mM at room temperature, of ∼10 mM at either physiological temperature or applying electrostimulation, and higher than 12 mM when both physiological temperature and electrostimulation were applied.

Topic: Tissue-Specific Focus

Subtopic: Cardiovascular tissues

Type: Poster presentation

TERMIS25_1519 Omnics analysis of progenitor cells in the developing human heart. Shannon Layland[1 2 3], Simone Liebscher[1], Katja Schenke-Layland[1 3]

[1]Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Tuebingen, Germany; *[2]Department of Women's Health Tübingen, Tuebingen, Germany*; *[3]NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

Introduction/Objectives

Human development is a potential blueprint for the identification and differentiation of progenitor cells for regenerative medicine application. In the cardiovascular system, multiple cell markers have been shown to identify cardiovascular progenitor cells (CPCs) in human development and have been used in differentiation protocols to derive CPCs from embryonic and induced pluripotent stem cells. However, some of these CPC markers may be progenitor cell markers in other organs and biological systems in the body. Interestingly, these markers are in similar locations within the developing heart, such as the outflow tract, making the differentiation between CPCs and non-CPC progenitor cells in the heart very important. In this study, we sought to differentiate CPC and non-CPC progenitor cells in the developing human heart.

Methods

Immunofluorescence staining (IF), NGS and proteomics of cardiac progenitor cells in first and second trimester human hearts from elective terminations was performed. Omnics data was analyzed using the Ingenuity Pathway Analysis (IPA) and the Gene Ontology Resource (GO) platforms.

Results

IF identified CPCs colocalized in three different morphological unique microenvironments. Omnics analysis demonstrated that the CPC microenvironments also where positive for gene and protein markers for other biological systems.

Conclusions

Our study demonstrates that CPC markers also identify progenitor cells from other biological systems and therefore must be used with caution when performing genetic analysis of human CPCs in the developing heart.

Topic: Tissue-Specific Focus

Subtopic: Degeneration

Type: Poster presentation

TERMIS25_206 Noggin Expression in Human Nucleus Pulposus Cells: A Comparative Study of Degenerative and Healthy Intervertebral Discs. Shuimu Chen, Sebastian Bigdon[1], Sonja Häckel[1], Christoph Albers[1], Zhen Li[2], Benjamin Gantenbein[1 3]

[1]Department of Orthopedic Surgery & Traumatology, Inselspital, University of Bern, Bern, Switzerland; *[2]AO Research Institute Davos, Davos, Switzerland*; *[3]Tissue Engineering for Orthopedics & Mechanobiology (TOM), Bone & Joint Program, Department for BioMedical Research (DBMR), Faculty of Medicine, University of Bern, Bern, Switzerland*

Introduction

Low back pain (LBP) is a health issue that leads to considerable disability and socioeconomic burdens. The degeneration of the human intervertebral disc (IVD) is a key factor in the pathogenesis of LBP. During disc degeneration, the nucleus pulposus (NP), a critical component of IVD, undergoes significant alterations in structural composition and function. Recent studies have highlighted the importance of various genes in IVD health. Noggin has been described as an essential gene in nucleus pulposus cells (NPCs) and a central role in inhibiting osteogenesis. In this study, we conducted a comparative analysis of Noggin expression in human NPCs from degenerative and healthy IVD.

Methods

Twenty-six IVD samples (nineteen from degeneration and seven from trauma) were collected to isolate NPCs for RNA extraction. Noggin expression was quantified at the transcript level by qPCR to analyze the difference between degenerative and healthy IVD.

Results

Results indicated that Noggin expression was lower in NPCs from degenerated IVD compared to trauma cases. The severity of IVD degeneration correlated with decreased Noggin levels. Healthy males exhibited

higher Noggin expression than healthy females and those with IVD degeneration. Noggin expression peaked in young adults (18-45y), decreased in middle age (46-69y), and rose again in the elderly (over 69y), but remained lower than in youth. Furthermore, upper lumbar discs (T12/L1-L2/3) showed higher Noggin levels than lower lumbar discs (L3/4/-L5/S1).

Conclusions

This research elucidates valuable insights into the variability of Noggin concerning sex, age, degree of disc degeneration and disc location. It provides implications for IVD health and LBP treatment.

Topic: Tissue-Specific Focus

Subtopic: Fibrosis

Type: Poster presentation

TERMIS25_1070 A multi-dimensional analysis of nintedanib and bosentan in the development of traumatic arthrofibrosis. Erik Wegner[1], Andreas Baranowski[1], Ulrike Ritz[1], Erol Gercek[1], Victoria Buschmann[1]

[1]*University Medical Center Mainz, Mainz, Germany*

Introduction/Objectives

An increasingly comprehensive understanding of both common and organ-specific mechanisms underlying the pathophysiology of fibrotic diseases has facilitated the development of highly potent antifibrotic agents. Nintedanib (NI) a tyrosine kinase inhibitor and bosentan (BO) a endothelin receptor antagonist have become key players in the treatment of various forms of pulmonary fibrosis. Arthrofibrosis (AF), another fibrotic disease that also shares significant genetic and functional ontological similarities with pulmonary fibrosis, lacks a potent pharmacological alternative. The translation of these already approved antifibrotic drugs to the treatment of arthrofibrosis could revolutionize conventional therapy, which relies on lengthy mechanical interventions with uncertain outcome.

Methods

In this double-blind study, thirty-nine Sprague-Dawley rats were randomly divided into three groups: NI, BO and placebo (PBO), each consisting of an equal number of animals. Following a standardised hyperextension injury of the right knee, the joints were immobilised at a 45° flexion angle for two weeks to allow joint contracture to develop. The uninjured left knee joints of the PBO group were used as a control group (n=13). During this immobilisation period, NI (2x2.5mg/kg/d), BO (2x250mg/kg/d) or PBO (2x250mg/kg/d) was administered orally. Joint contracture was assessed multi-dimensionally by measuring the extent of contracture at the biomechanical level using a newly developed automated precision arthrometer, analysing the cellular composition of the posterior joint capsule histologically using alpha-smooth muscle actin (*a*SMA) IHC, and measuring the mRNA expression levels of profibrotic genes.

Results

In contrast to BO, NI reduced *a*SMA mRNA expression 12-fold compared to placebo (p=0.0027, 2-way ANOVA), indicating a reduced fibroblast to myofibroblast transition of fibroblasts. However, as shown by comparable TGF*b* levels in all groups, NI did not completely stop fibrosis. Furthermore, comparable IL-6, N*f*kB, CTGF and TGF*b* values in the PBO and control group indicate that the acute phase of fibrotic formation must have occurred at an earlier stage than 2 weeks. A partial inhibition of fibrosis formation was also observed at the histological level, as a lower relative number of myofibroblasts tended to be confirmed under NI (p=0.08, Mann-Whitney test). Again, BO had no effect on the cellular composition of the joint capsule. Likewise, significantly greater joint mobility was observed in the NI group (80.56°) at 35 Nmm of torque (PBO = 63.85°, $p < 0.01$, Mann-Whitney test), whereas BO (63.36°) did not result in any advantage in joint mobility. The dynamic measurement of the force required to extend the flexed knee joint also

showed a significant advantage for NI over BO and PBO in a range of motion of 60°-90° ($p < 0.05$ multiple t-test).

Conclusions

This is the first experimental set-up to be described in the literature to investigate the use of NI in the development of traumatic arthrofibrosis. In contrast to BO, NI is able to slow down arthrofibrosis at comparatively low doses. Further studies should investigate higher concentrations and the local application of intra-articular drug scaffolds.

Topic: Tissue-Specific Focus

Subtopic: Fibrosis

Type: Poster presentation

TERMIS25_1436 Multi-pathway inhibition in the formation of arthrofibrosis: The combination of bosentan and nintedanib. Erik Wegner[1], Victoria Buschmann[1], Andreas Baranowski[1], Ulrike Ritz[1], Erol Gercek[1], Philipp Drees[1]

[1]*University Medical Center Mainz, Mainz, Germany*

Introduction/Objectives

Targeting cellular pathways could replace current standards of care for post-traumatic arthrofibrosis (PTA), which rely on prolonged mechanical interventions with uncertain outcomes. Recently, we were able to demonstrate the antifibrotic effect of nintedanib (NI), a small molecule tyrosine kinase inhibitor approved for various forms of pulmonary fibrosis, on the development of PTA in an animal model for the first time. This efficacy is likely due to the shared signaling pathways and overlapping fibroblast clusters involved in both conditions. In order to potentiate the antifibrotic effect of NI in our PTA model, a possible pro-inflammatory bypass pathway should be eliminated by bosentan (BO), a competitive endothelin A/B receptor antagonist with robust antifibrotic properties.

Methods

This double-blind study involved thirty-nine Sprague-Dawley rats, which were randomly assigned to one of three groups: NI, NI + BO, and placebo (PBO), with each group containing an equal number of animals. Following a standardized hyperextension injury to the right knee, the joints were immobilized at a 45° flexion angle for a period of two weeks to allow the development of complete joint contracture. The uninjured left knee joints of the PBO group served as the control group (n=13). During the immobilization period, the groups received oral administration of NI (2x2.5 mg/kg/day), NI + BO (2x2.5mg/kg/d + 2x250mg/kg/d), or PBO (2x252.5 mg/kg/day). The extent of joint contracture was assessed using a multifaceted approach: biomechanically, with a newly developed automated precision arthrometer to measure the extension deficit caused by contracture; histologically, by examining the cellular composition of the posterior joint capsule through alpha-smooth muscle actin (aSMA) immunohistochemistry (IHC); and at the molecular level, by quantifying the mRNA expression levels of profibrotic genes.

Results

At the biomechanical level, NI (80.56°) and NI+BOS (81.07°) significantly increased joint mobility compared to PBO(63.58°), indicating reduced contracture formation ($p<0.05$, multiple t-test). However, the drug combination did not show a measurable advantage over monotherapy with NI. A similar trend was observed in the expression of pro-inflammatory genes in the posterior joint capsule, with the NI and NI+BO groups showing approximately the same 12-fold reduction in a-SMA expression ($p<0.01$, 2-way ANOVA). This suggests a comparable reduced transition from fibroblasts to myofibroblasts. However, as the comparable TGF*b* levels in both groups show, neither the combination therapy nor the monotherapy was able to completely stop the development of the PTA. The incomplete inhibition of fibrosis formation was also confirmed at the histological level, as the relative number of myofibroblasts tended to be lower in the NI

and NI+BO groups compared to the PBO group (p=0.08, Mann-Whitney test). Again, no advantage was seen between the drugs.

Conclusions

The additional inhibition of other profibrotic signalling cascades by BO does not show an advantage over NI monotherapy. It can be assumed that the demonstrated antifibrotic effect is due to the sole effect of NI.

Topic: Tissue-Specific Focus

Subtopic: Fibrosis

Type: Poster presentation

TERMIS25_1507 Preventing Vocal Fold Fibrosis and Promoting Airway Functional Recovery after Intubation Trauma Treated with Composite-Coated Endotracheal Tubes. Gabriela Cervantes Gonzales[1], Ronit Malka[2], Joo Ong[1], Rena Bizios[1], Gregory Dion[3], Teja Guda[1]

[1]University of Texas at San Antonio, San Antonio, United States; *[2]Brooke Army Medical Center, Fort Sam Houston, United States*; *[3]University of Cincinnati Medical Center, Cincinnati, United States*

Introduction/Objectives

Endotracheal intubation is a common clinical procedure to maintain airway patency, however, it frequently results in laryngeal injury. Patients often experience discomfort with speaking, swallowing, and breathing in the initial recovery period. Unfortunately, clinical evaluations typically occur long after intubation, by which time acute tissue injuries may have progressed to fibrosis, leading to glottic or subglottic stenosis that requires surgical intervention. Developing therapeutic strategies for the acute phase of wound healing could minimize scar tissue formation and preserve vocal fold function. This study aimed to evaluate the efficacy of a dual-drug-eluting endotracheal tube (ETT) with a composite coating for targeted delivery of corticosteroids and siRNA targeting SMAD3, designed to immediately reduce pro-fibrotic TGFβ-1 signaling.

Methods

Traumatic intubation was simulated in swine under direct laryngoscopy. ETTs were coated with dexamethasone-loaded polycaprolactone (PCL) fibers via electrospinning, followed by embedding in a four-arm polyethylene glycol acrylate (4APEGA) matrix containing siRNA targeting SMAD3. Composite-coated, dexamethasone only, and uncoated ETTs were placed in the injured airway for 3, 7, or 14 days (n = 3 per ETT type per timepoint). Local mechanical properties post-injury and ETT placement were evaluated via normal indentation testing. Airway inflammatory cytokines were quantified by immunoassay, and laryngeal tissue was immunostained for CD86 and CD206 to assess macrophage polarization. Airway microbiome changes were analyzed using 16S rRNA sequencing, while biofilm formation was visualized by scanning electron microscopy (SEM) and micro-computed tomography (μCT). MUC5AC, a key mucin component, was measured via enzyme-linked immunosorbent assay (ELISA).

Results

Local stiffness was significantly higher in composite-coated ETTs at 3 days compared to uncoated ETTs. By 7 and 14 days, stiffness was greater in tissues with uncoated ETTs. IL-4 levels were significantly lower in composite-coated ETT groups compared to dexamethasone-only groups at 3 days and regular ETT groups at 7 days. Macrophage polarization showed an increased presence of CD86-positive (M1) macrophages in the composite-coated group at 3 days. Biofilm volume was higher in composite-coated ETTs, with significantly greater accumulation by 14 days. SEM confirmed high bacterial abundance. Airway microbiota composition was significantly influenced by both injury and ETT type. MUC5AC levels showed no significant differences, but mucin levels in composite-coated ETTs were closest to native control tissue across all timepoints.

Conclusions

Dual-drug delivery using composite-coated ETTs reduced vocal fold stiffness over 14 days of intubation compared to uncoated ETTs, suggesting a potential restoration of mechanical properties toward native tissue characteristics. Composite-coated ETTs significantly reduced anti-inflammatory cytokines, such as IL-4, and promoted a shift toward a pro-inflammatory M1 macrophage phenotype. However, these benefits were accompanied by increased biofilm formation and bacterial colonization compared to uncoated ETTs. Importantly, no evidence of mucus hypersecretion was observed, as MUC5AC levels remained comparable across groups. These findings highlight the potential of composite-coated ETTs to modulate inflammation and restore mechanical outcomes.

Topic: Tissue-Specific Focus

Subtopic: Gastrointestinal system

Type: Poster presentation

TERMIS25_527 3D bioprinting of colorectal cancer organoids using tissue mimetic bioink for drug screening and precision medicine applications. Madhumathi Rajendran[1], Dhakshinamoorthy Sundaramurthi[1], Swaminathan Sethuraman[1]

[1]*SASTRA Deemed University, Tirumalaisamudram, Thanjavur, India*

Introduction/Objectives

Organoids, a three-dimensional organ like mimics are emerging as a powerful tool in the area of the research for drug screening and personalized medicine applications. In the last few years, researchers have been developing strategies to combine conventional tissue engineering methods with biofabrication to develop next generation 3D tissue/organ models. This convergence enables the precision in controlling the spatial distribution of matrices, reproducibility, printing models with desired geometries & colorectal tissue topographies that are ideal requisites to recapitulate the native functionalities. Several hydrogel matrices were successfully utilized to make colorectal tissue constructs via conventional hydrogel 3D culture method as well as biofabrication methods. However, the developed tissue models do not fully replicate the native biological and physical properties of colorectal cancer tissue. To overcome this challenge, several research groups have been focusing of the development of an ideal hydrogel bioink for the fabrication of colorectal cancer model by using a novel combination of biomaterials with suitable crosslinking strategies. However, no ideal bioink hydrogel system exists towards the development of colorectal cancer *in vitro* models with properties matching the native tissue. To address this challenge, we have developed a protein-polysaccharide based hydrogel bioink which mimics the physical properties of native colorectal cancer.

Methods

The rheological analysis was performed for the developed GL/κC bioink to assess their thermoresponsive behaviour, viscoelastic properties and gelation kinetics. The optimised GL/κC bioink was bioprinted with caco2 cells in form of droplets using volumetric based bioprinting system and then crosslinked with microbial Transglutaminase (mTG). Further, the cell viability and proliferative assay was performed to assess the cytocompatibility of cells in the bioprinted GL/κC constructs. In addition, cytoskeletal staining was carried out to determine the organizaiton of caco2 cells in the spheroids.

Results

Initially, the bioink composition was optimized based on the gelation kinetics and the viscoelastic behaviour suitable for 3D volumetric bioprinting applications. To improve the stability and mechanical properties of the printed constructs, a cytocompatible enzymatic crosslinking was carried out using mTG. The mTG crosslinking of 3D bioprinted GL/κC bioink enhanced the stability of the hydrogel constructs under physiological conditions and offered appropriate mechanical strength closely matching the colorectal cancer tissues. Further, 3D constructs bioprinted with human intestinal epithelial cell (Caco2 cells) exhibited excellent viability and proliferation. Moreover, the cytoskeletal staining at day 14 revealed that the bioink

significantly favored cell-cell interaction and polarization of cells that are vital for intestinal lumen development. Based on the above results, it may be concluded that the developed GL/κC bioink has a promising potential to be used for the biofabrication of colorectal 3D tissue models.

Conclusions

We have developed a hybrid 3D bioprintable hydrogel bioink by combining gelatin (GL) & κ-carrageenan (κC). These biofabricated colorectal 3D cancer models could also serve as an efficient platform for high throughput drug screening and precision medicine applications.

Topic: Tissue-Specific Focus

Subtopic: Kidney

Type: Poster presentation

TERMIS25_1144 Organized tubular scaffolds to build vascularized kidney proximal tubules.

Anne Metje van Genderen[1], Essa Al-Jehani[1 2], Sebastian Beusink[1], Thibault Sampon[1], Marta Garcia Valverde[1], Jos Malda[3 4], Tina Vermonden[5], Jitske Jansen[6], Mylene de Ruijter[3 4], Silvia Mihăilă[1], Miguel Castilho[2 7], Roos Masereeuw[1]

[1]Department of Pharmaceutical Sciences, Pharmacology, Utrecht University, Utrecht, Netherlands; *[2]Department of Biomedical Engineering, Technical University of Eindhoven, Eindhoven, Netherlands*; *[3]Department of Orthopaedics, University Medical Center Utrecht, Utrecht, Netherlands*; *[4]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands*; *[5]Department of Pharmaceutical Sciences, Pharmaceutics, Utrecht University, Utrecht, Netherlands*; *[6]Division of Nephrology and Clinical Immunology, Medical Faculty RWTH Aachen University, Aachen, Germany*; *[7]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands*

Introduction/Objectives

Kidney tubular engineering requires structures of small sizes (≤1mm) to mimic curvature of native kidney proximal tubule (PT), which is key for cellular functionality and maturation. Here, we created highly porous rhombus tubules using melt-electrowriting (MEW) to build a vascularized proximal tubule.

Methods

A custom-built MEW device was used to fabricate small polycaprolactone tubular scaffolds (inner ø: 1 mm) with defined rhombus microarchitectures (winding angles of 30°, 50° and 70°). Human conditionally immortalized proximal tubule epithelial cells (ciPTEC) and glomerular endothelial cells (ciGEnC) were seeded alone or consecutively in the scaffolds and evaluated via immunofluorescent stainings for monolayer formation and tightness (zonula occludens-1, cluster of differentiation 31 (CD31)), cell directionality (F-actin), polarization (α-tubulin, Na^+K^+-ATPase) and extracellular matrix (ECM) production (collagen IV). Furthermore, gene expression of endothelial (CD31 and Von Willebrand Factor) and PT (organic cation transporter-2 (OCT2), P-glycoprotein (P-gp), multidrug resistance-associated protein 4 (MRP4)) markers was measured via quantitative reverse transcription polymerase chain reaction, and P-gp transport was studied in the tubular scaffolds.

Results

Engineered tubular scaffolds (length: 2 cm, inner ø: 1 mm) with different rhombus microarchitectures were successfully manufactured by controlling key instrument parameters. Both ciPTEC and ciGEnC formed tight, polarized monolayers within the tubular scaffolds and generated collagen IV-enriched ECM. The 30° winding angles induced preferential cell alignment along the scaffold fiber direction for ciPTEC and ciGEnC and facilitated formation of polarized monolayers. Consecutive seeding of first ciPTEC and, after monolayer formation, ciGEnC enabled growth of endothelial cells on the ECM deposited by ciPTEC, thereby forming a vascularized PT, maintaining high gene expression of PT markers (OCT2, P-gp, and MRP4), and showing functionality of P-gp in co-culture.

Conclusions

MEW tubes with highly controlled fibrous microarchitectures advance both ciPTEC and ciGEnC cell organization and ECM deposition. Our results show the first prototype of a vascularized scaffold, where the basement membrane formed by ciPTEC supports ciGEnC adhesion and growth. This innovative approach not only shows the potential of MEW in tissue engineering but also sets a new benchmark for the creation of functional, bioengineered kidney models.

Topic: Tissue-Specific Focus

Subtopic: Kidney

Type: Poster presentation

TERMIS25_1266 Enhanced kidney decellularization after pretreatment with high hydrostatic pressure. Haitham Salti[1], Jacqueline Heskamp[1], Anne Breitrück[1], Steffen Mitzner[2], Reinhold Wasserkort[1]

[1]Fraunhofer Institute for Cell Therapy and Immunology (IZI), Rostock, Germany; *[2]Rostock University Medical Center, Rostock, Germany*

Introduction/Objectives

The optimization of kidney decellularization protocols is critical for advancing kidney tissue engineering, which holds significant promise for addressing the shortage of donor organs. In our previous studies, high hydrostatic pressure (HHP) treatment followed by perfusion decellularization using chemical reagents demonstrated limited efficacy, with a comparatively low reduction in residual DNA. Histological analysis revealed evidence of HHP-induced tissue compression, suggesting vascular network constrictions as a potential barrier to effective chemical perfusion. This study aimed to optimize the perfusion decellularization process following HHP treatment.

Methods

The effect of HHP on collagen denaturation was assessed using fluorescently-labeled collagen hybridizing peptide (F-CHP), which binds to denatured collagen. Rat tendons were subjected to HHP at 420 MPa, and the results were compared to tendons treated with 1% SDS or 1% Triton X-100. Following treatment, the tendons were stained with F-CHP. Moreover, we investigated two distinct approaches for kidney decellularization following HHP treatment: direct and indirect decellularization. In the direct approach, kidneys were pretreated with HHP at 70 MPa, then perfused with 144 mL of 1% sodium dodecyl sulfate (SDS) for 6 hours, followed by a washing step with 350 mL of PBS for 14.5 hours at a flow rate of 0.4 mL/min. In contrast, the indirect approach involved pretreatment with HHP at 105 MPa, followed by preconditioning through perfusion with 432 mL of PBS for 18 hours to alleviate potential vascular constrictions caused by HHP. Subsequently, kidneys were perfused with 144 mL of 0.5% SDS for 6 hours and washed with 350 mL of PBS for 14.5 hours at the same flow rate. The effectiveness of these protocols was assessed by quantifying residual DNA content and glycosaminoglycan (GAG) preservation in the extracellular matrix (ECM). Histological analysis using hematoxylin and eosin staining was also conducted to evaluate the integrity of the ECM strucutre.

Results

F-CHP analysis revealed that 1% SDS induced significant collagen denaturation 10 times higher than Triton X-100. In contrast, HHP at 420 MPa induced negligible collagen denaturation, highlighting its mild effect on ECM integrity. Direct decellularization using 1% SDS achieved a 24% reduction in DNA content, while pretreatment with HHP (70 Mpa) followed by decellularization with 1% SDS led to only a 6% reduction, indicating a highly ineffective decellulariztion. In contrast, indirect decellularization introduced a preconditioning step involving PBS perfusion after HHP treatment (105 Mpa) and before decellularization with 0.5% SDS. This approach significantly improved decellularization outcomes, achieving a 64%

reduction in DNA content despite the lower SDS concentration but also led to an approximate 78% reduction in GAG content.

Conclusions

Introducing a PBS preconditioning step significantly enhanced perfusion decellularization following HHP treatment, improving DNA removal while reducing the required SDS concentration. Although HHP minimally affected collagen integrity, vascular compression hindered decellularization without preconditioning with PBS. The indirect approach addressed these challenges but resulted in reduced GAG content, highlighting the need for further optimization to balance decellularization efficacy with ECM preservation.

Topic: Tissue-Specific Focus

Subtopic: Liver

Type: Poster presentation

TERMIS25_831 3D Bioprinting of Humanized Xeno-free Liver Models. Ahmed Ali[1], Dongwei Wu[1], Johanna Berg[1], Viola Roehrs[1], Johannes Hackethal[2], Lisa Woelken[1], Cornelia Rauh[1], Albert Braeuning[3], Jens Kurreck[1]

[1]Technische Universität Berlin, Berlin, Germany; *[2]THT Biomaterials, Vienna, Austria*; *[3]German Federal Institute for Risk Assessment (BfR), Berlin, Germany*

Introduction/Objectives

Significant efforts are being made to develop new approach methods (NAMs) to replace animal experiments. However, most of the reported NAMs, including 3D bioprinted cell culture models, involve the use of animal-derived components.

Methods

The present study aims to develop bioprinted liver models that are completely devoid of animal components. As proof-of-concept, a xeno-free liver model was developed based on HuH-7 cells [1]. Cells were adapted to a chemically defined medium (CDM) using both direct and sequential approaches. Furthermore, three different chemically defined freezing media were prepared and optimized for the cryopreservation of adapted cells. Additionally, a xeno-free bioink was formulated based on sodium alginate, human collagen (I), and nutrient supplements to avoid the use of animal components in bioprinting. The

Results

The resulting xeno-free bioprinted liver models demonstrated high cell viability and metabolic activity, comparable to that of the Matrigel-based liver model. To assess the applicability of this xeno-free model, it was used to test the hepatotoxicity of okadaic acid, and results were compared to conventional 2D cell culture. To further improve the model, the established CDM was optimized to grow additional cell lines, including HepaRG (hepatic), LX-2 (hepatic stellate), and HUVEC (endothelial) cells and THP-1 cells.

Conclusions

The co-culture xeno-free model can thus be used as a potential alternative to animal testing for high-throughput hepatotoxicity evaluation.

Topic: Tissue-Specific Focus

Subtopic: Liver

Type: Poster presentation

TERMIS25_921 Non-perfusion decellularization of porcine and human liver – searching for ideal scaffold for artificial liver. Maria Stefania Massaro[1], Richard Palek[1 2], Ilya Matsviayonak[1], Ekaterina Panova[1], Marek Brousil[1], Lenka Cervenkova[1], Jan Sevcik[1 2], Vaclav Liska[1 2], Vladimira Moulisova[1]

[1]*Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic*; [2]*Department of Surgery, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic*

Introduction/Objectives

Considering the shortage of available liver organs compared to the demand from patients waiting for a transplant, new ways to provide a functional replacement are being investigated. The current procedure is to use an allogeneic organ from another human donor to minimize risks related to cross-species rejection, but the number of available donors is limited. To solve this issue, the idea is to use xenogeneic donors after removing the responsibles of the immune rejection. In the last two decades the decellularization has emerged as a technique to provide a non-immunogenic scaffold which keeps the morphology of the native organ and should enhance a healing response. Here, we aim to compare the human liver with the porcine counterpart at the microstructure level as well as the ability of the scaffold to be repopulated with human cells *in vitro*.

Methods

Small human livers pieces were obtained from the Department of Surgery of Pilsen University Hospital, porcine liver pieces were obtained during experiments performed at the Biomedical Center. All procedures were performed in line with corresponding ethical approvals. Small pieces of 8 mm diameter were cut out from a frozen tissue with a biopsy punch. Decellularization was performed by agitation on a magnetic stirrer in a combination of cycles containing 4% Triton X-100 followed by 0.1% ammonium hydroxide in deionized water. Samples were then analyzed by histological and immunofluorescence (IF) stainings for overall quality and preservation of specific proteins, DNA residues content and glycosaminoglycans (GAGs) preservation. A static seeding of HepG2s cells was performed to verify the repopulation potential of both human and porcine scaffolds.

Results

The decellularization was effective for both human and porcine liver pieces, which overall appeared decellularized. However, we observed differences when the human livers were affected by steatofibrosis. Despite clear reduction of DNA content in the scaffolds and the absence of nuclei in the haematoxylin-eosin staining, residues of cells cytoskeleton were found after Cytokeratin-18 staining in both human and porcine pieces. As evidenced by IF, major proteins are preserved after the decellularization, although there is a significant loss of GAGs. After static *in vitro* repopulation, HepG2 cells were still active in scaffolds of both species after a week long culturing.

Conclusions

Non-perfusion decellularization works well for the preparation of small-sized scaffolds from healthy livers of both, human and pig. Nevertheless, the decellularization seems to be affected by the amount of fibrotic tissue in the liver parenchyma. Still, scaffolds from healthy tissues have most of the tested extracellular structures well preserved, and both types of scaffold support growth of human cells *in vitro*.

Financial support: Authors acknowledge support from project “Surgical disciplines” (COOPERATIO-207043) of Charles University, project AZV NU22J-06-00058 of the Ministry of Health of the Czech Republic, European Regional Development Fund - Project “Application of Modern Technologies in Medicine and Industry“ (No. CZ.02.1.01/0.0/0.0/17_048/0007280) and project National Institute for Cancer Research – NICR (EXCELES, n. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Topic: Tissue-Specific Focus

Subtopic: Liver

Type: Poster presentation

TERMIS25_1264 Integrative treatment approach for acute liver toxicity using Andrographis paniculata and stem cells therapy. Kapil Kumar Malviya[1], Anurag Pandey[1]

[1]*Institute of Medical Sciences, Banaras Hindu University, Varanasi, India*

Introduction / Objective: Being the main centre of metabolic activities, liver is more prone to injury specifically drug-induce leading to hepatic injury. Various hepatotoxic substances and free radicals of oxidative reactions negatively affect various proteins of the body through oxidative stress. Conventional therapies for liver damage often have limited efficacy and potential side effects. Hence it becomes important to explore safe and effective options to treat liver toxicity. Over the past few decades, it has been recognized that the natural phytoconstituents from plants help in the improvement of cell life and stability. *Andrographis paniculata* is a plant from Acanthaceae family. It has several biological properties including hepatoprotective effect, anti-inflammatory effect and anti-cancerous. In modern cell-based interventions, stem cells have emerged as a treatment approach for various conditions due their enormous therapeutic and differentiating capabilities. The present work aims to explore the combined therapeutic effect of modern medicine (mesenchymal stem cells (MSCs)) along with ethnomedicine (*A. paniculata* extract) in liver toxicity.

Objectives

1) Procurement of materials, *Andrographis paniculata* extract execution and isolation of mesenchymal stem cells from rat bone marrow.

2) In-vitro evaluation of *Andrographis paniculate* extract and MSCs for their therapeutic potential in CCl_4-induced hepatotoxicity.

3) In-vivo evaluation of *Andrographis paniculate* extract and MSCs for their therapeutic potential in CCl_4-induced hepatotoxicity.

Methods

A. paniculata extract was taken from plant leaves using standard procedure. Isolation of MSCs from rat bone marrow was performed. Effect of plant extract and MSCs was first checked in-vitro using liver derived cell lines by creating liver toxicity model both individually and in combination. Cell viability assay, proliferation assay and other parameters were checked in-vitro. Post cell work in-vivo analysis was done by administering *A. paniculate* extract and MSCs in the rats subjected to CCl_4 inducing hepatotoxicity. The liver function tests were done to detect the liver function with the help of a biochemical analyzer. Histology of the liver in all the groups was checked by using hematoxylin and eosin staining.

Result

Both in-vitro and in-vivo results showed protective role of *A. paniculate* alone and in combination with MSCs in liver toxicity. During the in-vitro work, plant extract showed no toxicity on hepatocytes. Good proliferation, migration and viability was seen on cells plant extract and combinatorial treatment in the cell toxicity model. During the in-vivo studies, in treatment group with the plant extract along with MSCs in CCl_4 induced toxicity model, the animals showed improved liver function tests and healthy liver morphology after sacrifice as compared to CCl_4 group. Histology studies also showed improved cell morphology and ultrastructure in the combinatorial treatment group.

Conclusion

The hepatoprotective role of *A. paniculate* in combination with MSCs in liver toxicity opens new horizons for improvised and safe medicinal options for liver regeneration and protection. The effectiveness of this treatment approach can further be improved through recognition of active compounds in *A. paniculate* and amalgamating them with advanced tissue engineering approaches.

Topic: Tissue-Specific Focus

Subtopic: Lung

Type: Poster presentation

TERMIS25_1344 Application of uniaxial, cyclic stretch to a 3D airway mucosa equivalent at air–liquid interface using a textile-fibrin composite. Hannah Kubiza[1 2], Julian Gonzalez-Rubio[1], Christian Cornelissen[1], Stefan Jockenhoevel[1], Jens Konrad[3], Bernd Hoffmann[3], Rudolf Merkel[3], Anja Lena Thiebes[1]

[1]*Department for Biohybrid & Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, RWTH Aachen University, Aachen, Germany*; [2]*Institute of Molecular and Cellular Anatomy (MOCA), RWTH University Hospital Aachen, Aachen, Germany*; [3]*Forschungszentrum Jülich, Institute of Biological Information Processing, IBI-2: Mechanobiology, Jülich, Germany*

Introduction/Objectives

Within the respiratory system, the airways are lined with a highly specialized mucosa, which consists of a pseudostratified epithelium and its lamina propria, a layer of loose connective tissue. The epithelium is mainly comprised of three cell types: ciliated, secretory and basal cells. The mucosa enables important barrier functions by clearance of mucus-entrapped particles and pathogens from the airways through unidirectional, coordinated ciliary beating. Within the respiratory system, mechanical forces like wall sheer stress, stretch and compression caused by physiological processes such as breathing or pathological conditions such as coughing or bronchial constriction are omnipresent. Especially stretch has been shown to influence biological processes like cellular differentiation, alignment and synthesis of extracellular matrix proteins. However, most current airway *in vitro* models neglect mechanical forces as an important factor to guide mucosal maturation and functionality.

Methods

The presented approach utilizes a three-dimensional co-culture model of the bronchus consisting of primary human respiratory epithelial cells seeded onto a fibrin hydrogel enriched with bronchial mesenchymal cells. To allow simultaneous cultivation at air-liquid interface and the application of uniaxial, cyclic stretch, the fibrin hydrogel is cast around a silicone-coated medical-grade PET textile. Cultures were maintained statically for 1 week, before application of a cyclic 5% strain amplitude either for 4 hours or up to 7 days using a frequency of 0.16 Hz. Respective static controls were maintained in identical cultivation conditions. Afterwards, tissue integrity is evaluated using (immuno)histochemistry and electron microscopy. Gene expression analysis is performed using quantitative PCR.

Results

Characterization of the unidirectional strain showed that homogenous strain distribution throughout the sample is dependent on the design and the geometry of the hydrogel-textile-silicone-composite. After 48 hours of cyclic strain, no stress relaxation of the materials was observed. Bronchial co-cultures were successfully maintained for up to 7 days under dynamic conditions and demonstrated an intact epithelial barrier formation. Moreover, mRNA and protein expression of airway differentiation markers showed different responses of the cells to short-term (4 hours) and long-term (7 days) stretching periods.

Conclusions

This novel airway *in vitro* system achieves new levels of complexity by enabling the cultivation of bronchial epithelial cells at air-liquid interface on a three-dimensional hydrogel scaffold, while applying a uniaxial, cyclic strain mimicking physiological breathing patterns. These results prove its usefulness to investigate the impact of mechanical forces and their mechanotransduction on tissue maturation and organization of the respiratory mucosa.

Topic: Tissue-Specific Focus

Subtopic: Lung

Type: Poster presentation

TERMIS25_1560 Engineering Pulmonary Scaffolds with mcl-PHA Polymers. Samruddhi Mujumdar[1], Annabelle Fricker[1], Syed Mohammed Daniel Syed Mohammed[1], Ipsita Roy[1 2]

[1]*University of Sheffield, Sheffield, United Kingdom*; [2]*Insigneo Institute of in silico medicine, University of Sheffield, Sheffield, United Kingdom*

Introduction/Objectives

The clinical burden of lung diseases underscores the urgent need for advanced disease models capable of replicating the native architecture and functionality of lung tissue. Three-dimensional (3D) scaffold-based *in vitro* models have emerged as a promising platform, addressing the limitations of traditional approaches by providing a physiologically relevant and reproducible environment.

Polyhydroxyalkanoates (PHAs) are a sustainable, FDA-approved family of polyesters produced via bacterial fermentation under nutrient-limiting conditions. Their inherent properties-low immunogenicity, biocompatibility, and tunability-make them suitable for biomedical applications. Medium-chain length PHAs (mcl-PHAs) have shown potential in soft tissue engineering, highlighting their promise in replicating the natural lung environment.

This study evaluates the application of mcl-PHAs to produce *in vitro* 3D pulmonary models by investigating their mechanical and structural properties and assessing the cytocompatibility of lung cells on both 2D and 3D mcl-PHA scaffolds.

Methods

Polymer was derived from a fed-batch fermentation of a Gram-negative bacterium, for 24 hours, in a stirred tank bioreactor. The fermentation broth was centrifuged to obtain a cell pellet, which was subsequently lyophilised. The dried biomass was subjected to a beaker extraction method to obtain pure polymer. Characterision of physical, chemical and thermal properties of a solvent-cast PHA film was carried out ahead of 3D printing. mcl-PHA scaffolds were then analysed via SEM. Tensile testing was used to understand the extent of similarity to lung tissue. Cytocompatibility testing, using a A549 lung adenocarcinoma cell line was carried out by seeding on 2D PHA films. Viability and metabolic activity were assessed through live/dead staining, and resazurin assay respectively. Functionality of the cells was assessed using lung specific markers and immunofluorescence measurements.

Results

The polymer produced was identified as a mcl-PHA, with GC-MS analysis detecting monomers ranging from C_6-C_{14}. FTIR spectra further confirmed its structure. Thermal analysis revealed a low melting point and high degradation temperature, demonstrating suitability for 3D printing.

Naturally, mcl-PHA is elastomeric with a low Young's modulus. Fused deposition modelling based melt extrusion printing was successfully optimised and 3D scaffolds were produced. Post-printing, the scaffolds exhibited enhanced elasticity, achieving a compressive modulus of 1.2 MPa, aligning closer to the properties of native lung tissue. SEM imaging of 3D printed scaffolds confirmed uniform scaffold morphology and dimensions.

A549 cells seeded on 2D sterile PHA films demonstrated high viability and metabolic activity in a 7-day resazurin assay. Functionally, 2D PHA films supported cell growth with expected markers for surfactant proteins (SP-C), tight junctions (ZO-1), and epithelial integrity (E-cad) expressed over 7 days.

Conclusions

Mcl-PHA was produced using bacterial fermentation and characterised in depth. This showed high suitability for 3D printing and the subsequent scaffolds were both reproducible and similar to native lung tissue. mcl-PHA was able sustain growth of A549 cells on a 2D film. Future work in developing a high-fidelity 3D *in vitro* lung model will involve similar studies using 3D printed scaffolds and optimising their structure for mature lung cell phenotypes.

Topic: Submit to SYMPOSIUM

Subtopic: Bioelectronic Interfaces in Tissue Engineering: Electroconductive biomaterials and bioelectronics for enhanced repair, regeneration and monitoring

Type: Poster presentation

TERMIS25_892 Laser-induced graphene as an electroconductive biomaterial for skeletal muscle tissue applications. Jesús Ordoño[1], Monsur Islam[1], Andrés Díaz Lantada[2], De-Yi Wang[1],

Mónica Echeverry Rendón[1]

[1]*IMDEA Materials Institute, Getafe, Spain*; [2]*Universidad Politécnica de Madrid, Madrid, Spain*

Introduction/Objectives

Laser-induced graphene (LIG) has aroused as a promising, facile and cost-effective technique for the fabrication of graphene materials. This study explores the potential of LIG as an electroconductive, biocompatible material for skeletal muscle tissue applications.

Methods

Polyimide sheets (PI) were irradiated with an infrared CO2 laser beam using a ULS Versa Laser 3.50 engraving machine. The resulting LIG material was characterized in terms of surface topology, contact angle and electrical conductivity, while the structure, organization and nature of the graphitic material was evaluated using X-ray diffraction (XRD) and Raman spectroscopy.

Mouse C2C12 myoblast cells were seeded on LIG material and their viability (live/dead assay) and cell activity (resazurin cell viability assay) was assessed after 1, 3 and 6 days. The myogenic differentiation of C2C12 was induced using low-serum conditions (5%) and assessed through RT-qPCR and immunofluorescence of myosin. Attachment, shape and alignment of cells on LIG was also evaluated using scanning electron and fluorescent confocal microscopy.

Results

Laser irradiation of PI resulted in a highly porous and fibrilar LIG structure with a thickness of around 25µm high. The carbon material produced was highly graphitic, with disordered and stacked multi-layer graphene as supported by XRD and Raman spectroscopy, consistent with previous reports on PI-derived LIG material. This resulted in a good electrical conductivity (5.8 S/cm) and a hydrophobic surface with a contact angle of 95.3°. Myoblast cells showed an excellent attachment and growth on LIG surface, with >95% of viability after 24h of culture and supporting cell proliferation after 6 days. The LIG material also supported myogenic differentiation of skeletal muscle cells, which showed expression of early and late myogenic markers such as Desmin, MyoD, MYH1 and Myogenin. Confocal and scanning electron microscopy images revealed a good adhesion of myosin-positive cells on the porous structure of LIG, displaying the characteristic elongated shape of skeletal myoblasts and a clear cell alignment following the directional linear pattern of the laser engraving, thus resembling the architecture of skeletal muscle tissue.

Conclusions

This study demonstrates the potential of laser-induced graphene as an electroconductive, biocompatible material for skeletal muscle applications, supporting proliferation and differentiation of myoblast cells and with the ability to mimic the native orientation of muscle tissue. These results open the possibility of using LIG-derived materials as bioelectronic interfaces and biomaterials for repair, regeneration or monitoring of skeletal muscle tissue.

Topic: Tissue-Specific Focus

Subtopic: Muscle

Type: Poster presentation

TERMIS25_1025 Two-photon 3D printing of synthetic scaffolds towards skeletal muscle regeneration. Theresa Kühn[1], André Tomalka[2], Tobias Siebert[3], Michael Heymann[4]

[1]*Institute of Biomaterials and Biomolecular Systems, University of Stuttgart, Stuttgart, Germany*; [2]*Institute of Motion and Exercise Science, University of Stuttgart, Stuttgart, Germany*; [3]*Institute of Motion and Exercise Science, University of Stuttgart, Stuttgart Center for Simulation Science, Stuttgart, Germany*; [4]*Institute of Biomaterials and Biomolecular Systems, University of Stuttgart, Stuttgart Research Center Systems Biology, University of Stuttgart, Stuttgart, Germany*

Introduction/Objectives

Two-photon 3D printing is a promising tool to fabricate bio-scffolds with micrometer resolution to replicate soft native microtissues in vitro. Desired scaffold architectures can be polymerized from protein-based bioinks, while mechanical properties of the printed structures can be set by adjusting print parameters. We seek to adopt this versatile and high-resolution 3D printing to replace damaged tissue and to promote tissue regeneration in vivo in the future.

Methods

We employed a simplified pH-dependent muscle system to replicate the biomechanics of the skeletal muscle tissue by 3D printing a hydrogel fiber made from bovine serum albumin. Using a custom microscale tensile strength device, we quantified force-length relations and elastic properties of these 3D printed protein muscle fiber models.

Results

The observed pH-dependent contraction of the synthetic muscle (non-activated at pH 7, activated at pH 4) resulted in active forces comparable to those in native skeletal muscles. Furthermore, manipulating the length of this synthetic muscle affected its force production, which follows a force-length relationship, similar to native skeletal muscles. Ultimately, two-photon crosslinked bioinks can replicate the biomechanical properties of native skeletal muscle tissue. Moving forward, we seek to replace physiological muscle fibers where myocytes align and regenerate to exert contractile forces. Therefore, we studied tissue alignment of C2C12 precursor myocytes in spatially confined 3D printed structures. All tested substrate topographies either induced nematic ordering or the formation of topological defects of nearby myocytes, demonstrating the importance of extracellular 3D cues on detailed microtissue physiology.

Conclusions

This paves the way for optimizing bioink formulations and designs towards 3D printing synthetic scaffolds for skeletal muscle regeneration.

Topic: Tissue-Specific Focus

Subtopic: Muscle

Type: Poster presentation

TERMIS25_1093 Evaluation of Myoblast Gene Expression, Proliferation, and Migration in Response to Mannose and Galactose Therapy in Congenital Muscular Dystrophy Patients. Maja Ledinski[1], Marija Zekušić[1], Marina Bujić Mihica[1], Lucija Ćužić[2], Karla Ostojić[2], Inga Urlić, Jan Homolak[3], Tamara Žigman[4], Tiha Vučemilo[1], Ivo Barić[5]

[1]Sestre milosrdnice University Hospital Center, Zagreb, Croatia; *[2]University of Zagreb, Faculty of Science, Zagreb, Croatia*; *[3]University of Zagreb, School of Medicine, Zagreb, Croatia*; *[4]University Hospital Centre Zagreb, Zagreb, Croatia*; *[5]University Hospital Centre Zagreb, Department of Pediatrics, Zagreb, Croatia*

Introduction/Objectives

Congenital muscular dystrophies are rare conditions that are very challenging to treat. These disorders are primarily caused by mutations in genes that affect muscle structure and function, leading to progressive muscle weakness. Dystroglycanopathies are a group of muscular dystrophies associated with abnormal glycosylation of α-dystroglycan (α-DG), a laminin-binding protein that plays a key role in linking the cytoskeleton to the extracellular matrix. Dysfunctions in α-DG lead to a dysregulation of mechanical support and stability during muscle contraction. Therefore, our goal was to explore whether treatment with mannose and galactose, key sugars in the glycan chains of α-DG, could benefit patients with dystroglycanopathy caused by mutations in the *POMT2* gene (Protein O-Mannosyltransferase 2).

Methods

Skeletal myoblasts were isolated from muscle biopsies of two patients with *POMT2*-related dystroglycanopathy and one control patient. Morphology, viability, population doubling time, and proliferation rates were evaluated during cell culture. Myoblasts were treated with a range of mannose and galactose (50-1000 μM) concentrations as well as combination of galactose and mannose (140 μM/1300 μM) over a 5-day period. RNA was then isolated to perform expression analysis of *DAG1*, *POMT2*, and *PPARGC1A* by qPCR. Additionally, to evaluate myoblast proliferation, morphology, kinetics, and single-cell tracking, we treated myoblasts with a combination of galactose and mannose and performed time-lapse holographic microscopy imaging during 5 days.

Results

Expression of *DAG1* and *POMT2* was generally lower in patient myoblasts compared to the control. While gene expression of *DAG1* and *POMT2* increased in control myoblasts with treatment, expression in myoblasts from muscular dystrophy patients changed only slightly. Regarding *PPARGC1A*, a gene involved in the transcriptional regulation of oxidative phosphorylation, patient myoblasts showed dramatically lower expression compared to the healthy control, with no significant effect from treatment. Real-time holographic imaging provided quantitative data on myoblast morphology, behavior, migration and formation of myotubes. Furthermore, single-cell tracking enabled us to monitor cell cycle progression and migration speed of individual myoblasts, providing valuable insights into myoblast responses to the treatment.

Conclusions

These findings provide new perspectives on glycosylation-targeted therapy for dystroglycanopathies and offer a clearer direction for the future exploration of potential therapeutic strategies.

Topic: Tissue-Specific Focus

Subtopic: Muscle

Type: Poster presentation

TERMIS25_1408 Optimizing geometry and interface stability to increase the contraction efficiency of engineered skeletal muscle tissue constructs. Aiste Balciunaite[1], Mike Michelis[1], Miriam Filippi[1], Pablo Paniagua[1], Oncay Yasa[2], Robert Katzschmann[1]

[1]ETH Zürich, Zürich, Switzerland; *[2]The Hong Kong University of Science and Technology, Guangzhou, China*

Introduction/Objectives Engineering contractile skeletal muscle tissues with larger tissue volumes remains an unsolved challenge in the field of muscle tissue engineering. Counterintuitively, the largest deformation of contractile muscle tissue constructs occurs from those made of single cell layers; bulk constructs are limited to sub-millimeter deformation. In this work, we aimed to solve this problem by engineering a system to optimize the efficiency of transferring the contraction force of a skeletal muscle tissue construct to a scaffold for visible movement. We focused on optimizing the geometry of the skeletal muscle tissue construct and the stable adhesion of living and non-living materials for efficient force transfer.

Methods Computational modelling confirmed that the optimal design to maximize movement is a bilayer shape with an inner active layer and passive outer layer. We developed a bilayer biofabrication method to stably integrate a distinct muscle tissue layer to a hydrogel scaffold. We formed a bilayered construct by integrating skeletal muscle tissue onto microgrooved hydrogel scaffolds. The hydrogel scaffolds were fabricated by xolographic volumetric printing using 15% PEGDA (M.W. 700) and 5% gelatin methacrylate. A myoblast containing hydrogel (Matrigel and gelatin methacrylate) was post-seeded onto the hydrogel scaffold. Constructs were grown and differentiated for two weeks. After differentiation, construct contraction behavior was characterized by electrical stimulation and video recording. Muscle tissue formation was probed by histology and H&E staining, and confocal imaging after staining with F-Actin, DAPI, and MyoHC.

Results We demonstrate stable integration of a muscle tissue layer with a hydrogel scaffold that withstands the forces of matrix remodeling and shrinkage resulting from myoblast differentiation to myofibers. Histological cross-sections show the interface and a transition from acellularized scaffold to highly cellularized muscle tissue reminiscent of the cellular transition in the myotendinous zone. Our bilayered platform reliably produces contractile constructs of size 15mm x 3.5 mm x 1mm, with deformations up to 6.6 mm. The constructs demonstrated a variety of motions enabled by the larger deformations, such as walking, swimming, and jumping. Finally, we demonstrate that our system can be used to probe muscle dynamics and the mechano-coupling between multiple distinct constructs by showing multi-unit assembly. Contraction analysis showed that the deformation is additive but there is no effect on the contraction time.

Conclusions By addressing the challenges of material integration of skeletal muscle constructs, we enabled millimeter-scale deformations of skeletal muscle tissue constructs. We envision that our bilayered platform will allow us to study and better understand the complex mechanobiology of functional skeletal muscle tissue formation and activity.

Topic: Tissue-Specific Focus

Subtopic: Nerves, neural tissues, nervous system

Type: Poster presentation

TERMIS25_888 ORGAN-SPECIFIC EXPRESSION DATA STEERS CHOICE OF LAMININ FOR IN VITRO STUDIES IN BIOLOGICALLY RELAVENT DRUG DISCOVERY AND DEVELOPMENT PLATFORMS.

Sandra Falci[1], Malin Kele[1], Therése Kallur[2], Boris Eleuteri[1], Zhijie Xiao[1]

[1]*BioLamina AB, Sundbyberg, Sweden*; [2]*BioLamina Inc, Cambridge, United States*

Introduction/Objectives

The Drug Discovery and Development (DDD) process incorporates multiple cell culture platforms to assess the safety and efficacy of lead compounds against a target for an indication. In recent years, with the advancement of stem cell research protocols, there has been shift in focus from 2D cell culture techniques to more complex patient-derived models. Human pluripotent stem cells (hPSCs) require an extracellular matrix (ECM) for maintenance, expansion, and differentiation; laminins, a large ECM protein family enriched within basement membranes of epithelial and endothelial tissues, are an essential part of stem cell niches. While laminins are increasingly implemented in hPSC research, standardised protocols using a biologically relevant ECM in DDD platforms have not been developed.

Methods

Protein and gene expression analysis of laminin isoforms in four distinct tissues in vivo and effect of laminins on different cell-types in vitro

Results

Thus, we assessed protein and gene expression patterns of laminin isoforms in four distinct tissues most commonly used in DDD: the heart, liver, kidney and central nervous system (*in vivo*), before translation to proliferation and expression experiments in specified cell-types on laminin substrates (*in vitro*). We found the expression pattern is largely tissue- and cell type specific, for example, in the kidney. We also show that providing the relevant laminin isoform *in vitro* leads to increased standardization and functionality. For example, when using laminin as a substrate in culture, 43-times more dopaminergic neurons and >80% functional cardiovascular progenitor cells (vs ~50% with competitor substrates), were observed. We also demonstrate how Biolaminins can be implicated in downstream microfluidic platforms to recreate the in vivo microenvironment when flow is introduced.

Conclusions

Taken together, we demonstrate tissue- and cell-specific expression of laminin isoforms. *In vivo* expression data can guide scientists to recapitulate the ECM microenvironment of a given cell or tissue to promote optimal in vitro conditions–an important consideration as we aim to create more biologically-relevant cell-based models to move away from animal models. By doing so, current limitations relating to differentiation, reproducibility, and maturity of PSC-derived cells can be addressed in more sophisticated DDD applications, such as organ-on-a-chip platforms.

Topic: Tissue-Specific Focus

Subtopic: Ocular tissues

Type: Poster presentation

TERMIS25_229 Optimizing Automated Differential Pressure in a Bioreactor for Corneal Tissue Engineering.

Ramon Guzman III[1]

[1]*University of the Pacific, Stockton, United States*

Introduction/Objectives

Over 2.2 billion individuals worldwide suffer from preventable vision impairment, emphasizing the need for innovations in ophthalmology. Combined with bioreactor culture, tissue engineering is promising for developing transplantable corneal tissues, offering an alternative to donor-derived transplants. This study developed an automated differential pressure-controlled bioreactor to cultivate rabbit corneal fibroblasts under precise intraocular pressure (IOP) conditions, mimicking the cornea's native biomechanical environment to promote fibroblast proliferation and viability.

Methods

We developed a custom bioreactor system using polyether ether ketone (PEEK) as the primary structural material, for its biocompatibility and strength. The system features automated pressure control, nutrient flow, and a flexible collagen-coated silicone membrane for cell growth. Rabbit corneal fibroblasts (RCFs) were seeded in the bioreactor and in collagen-coated (CC) and non-collagen-coated (NCC) 24-well plates as controls. The bioreactor was placed in a controlled environment, with sterile conditions and continuous media flow maintained by a peristaltic pump, while a Programmable Logic Controller regulated IOP for 4-7 days.

After the culture period, cell viability and proliferation were assessed through MTT assays, with additional Western blot analyses planned for further protein characterization. Imaging was performed using a Nikon Eclipse Ti-U microscope to observe cell confluence and morphology. Three trials per pressure setting will be conducted to ensure statistically significance.

Results

A 7-day dry run validated the pressure control system's stability (11.2–13.1 mmHg) for cell culture. These results underscore the system's reliability in creating a consistent mechanical environment essential for cell culture.

MTT assays revealed lower bioreactor viability (29,900 ± 976 cells) compared to 24-well controls (CC: 84,700 ± 3,200; NCC: 81,900 ± 8,900). COMSOL simulations predicted edge effects, with the bioreactor having a viable area of 1.04 cm^2 (59.68% of the total area) compared to the 24-well plate's 1.90 cm^2. Taking the bioreactor's well diameter and edge effects into account, theoretical cell counts were calculated at 46,290. This value shows a 35.41% difference from the experimental count, highlighting discrepancies likely influenced by differential pressure effects on cell growth. Future work will explore optimizing pressure parameters to improve cell growth outcomes.

Despite reduced confluence at the membrane edges, central regions demonstrated similar growth to control conditions, as microscopy images showed. This finding suggests that the bioreactor's pressure environment effectively supported corneal fibroblast growth.

Conclusions

Our findings validate the capability of our bioreactor to sustain corneal fibroblast growth comparable to conventional methods while maintaining precise IOP. The system's automation and pressure modulation make it a promising tool for studying the impact of elevated intraocular pressure on corneal cell behavior. We are expanding the pressure range tested and integrating additional stimuli, such as light, to replicate the corneal environment more comprehensively. This bioreactor technology holds significant potential for advancing corneal tissue engineering, offering an alternative to donor transplants, and advancing vision restoration worldwide.

Topic: Tissue-Specific Focus

Subtopic: Ocular tissues

Type: Poster presentation

TERMIS25_1030 Repurposing the vitreous humor from livestock waste as an injectable substitute for age-related syneresis. Makayla Fahmy[1], Said Mohamad El Turk[1], Shamma Al Dhaheri[1], Hessa Almesafri[1], Rowdha Alhashmi[1], Aishah Alzeyoudi[1], Suhail Almheiri[1], Haider Butt[1], Peter Corridon[1]

[1]*Khalifa University, Abu Dhabi, United Arab Emirates*

Introduction/Objectives

Age-related syneresis, characterized by the gradual breakdown of the vitreous humor (VH) within the eye, significantly impacts visual health, particularly in the elderly. The current clinical solution for this is either the injection of a gas bubble or a vitrectomy, where the liquefied vitreous is removed and replaced with saline or a synthetic material, such as silicone oil. Current substitutes are not effective long-term options and fail to adequately replicate the properties of this natural hydrogel, highlighting the need for better, more biocompatible alternatives. Thus, this study's primary and long-term objective is to generate an injectable vitreal substitute using repurposed natural and intact ocular hydrogels. Post-mortem vitreal gels are too viscous for injection, so we aimed to enhance their injectability through homogenization. Our secondary and immediate objective was to compare the optical properties of this hydrogel pre- and post-homogenization to examine optimal injectability across species and storage conditions, ultimately to generate a viable human.

Methods

The intact VH was extracted from traditional (cow and sheep) and exotic (camel) whole eyes and stored under fresh, short-term, and long-term conditions, corresponding to 2 hours post-mortem, stored at -20°C for 24 hours, and stored at -80°C for over 3 months, respectively. Following extraction, optical transmittance was measured. The samples were then homogenized, and injectability, via flow rate and clogging, was tested by infusing 1 mL of homogenized VH into 24-well plates using various needle gauges. Following this, optical transmittance within the visible spectrum was measured again to assess whether clarity was maintained post-injection.

Results

Transparency analysis of freshly extracted VH revealed consistent transmittance levels comparable to humans for all species within the 400-700 nm range. Moreover, manual homogenization rendered the VH injectable through 20-, 21-, and 25-gauge needles, with no visible clogging of the needle observed. The needle gauge that resulted in the greatest flow rate was 21G, which gave a flow rate of 0.0534 mL/s, although 25G, typically used in such clinical applications, was comparable with a flow rate of 0.0529 mL/s. Following homogenization and injection of fresh VH samples, we achieved optical transmittance levels high as 90-95%, with no significant differences observed between intact samples and homogenized samples.

Conclusions

Our initial findings highlight the potential to generate an injectable vitreal substitute model using repurposed ocular hydrogels from various animal sources. While the optical clarity of the homogenized and injected samples showed a slight reduction, the transparency remained above acceptable levels for clinically relevant consideration. This research highlights a first step in repurposing ocular waste as a viable and biocompatible alternative for vitreous replacement. Further detailed studies are required to optimize optical clarity, consistency, and the ability to integrate into the innate VH, enhancing the clinical viability of this approach.

Topic: Tissue-Specific Focus

Subtopic: Ocular tissues

Type: Poster presentation

TERMIS25_1441 Dynamic cultivation of ex vivo porcine corneas using a silicone flow bioreactor enhances tissue preservation and wound healing. Sabina Eibichova[1], Thomas Schwarz[2], Jan Hansmann[2 3], Christian Lotz[1 2]

[1]University Hospital Würzburg, Department for Functional Materials in Medicine and Dentistry, Würzburg, Germany; *[2]Translational Center for Regenerative Therapies, Fraunhofer Institute for Silicate Research, Würzburg, Germany*; *[3]Faculty of electrical engineering, University of Applied Science Würzburg-Schweinfurt, Schweinfurt, Germany*

Introduction/Objectives

Porcine corneas, which exhibit structural and functional similarities to human corneas, are the standard ex vivo models in ophthalmological research, enabling studies on wound healing, drug permeability and infection. Mechanical forces, such as shear stress from blinking and intraocular pressure, are critical for maintaining the integrity of ex vivo cornea models. Flow bioreactors that incorporate these forces during cultivation have been shown to improve corneal tissue preservation. However, these bioreactor systems are often produced using materials like polyether ether ketone (PEEK) or polysulfone (PSU), which require specialized equipment for fabrication. This can result in several challenges, including limited accessibility, extended production times, and restricted flexibility for design modifications. In this study, we developed a cost-effective silicone-based flow bioreactor for the dynamic cultivation of ex vivo porcine corneas.

Methods

The silicone flow bioreactor was fabricated with four components, including two main parts with attached upper and lower lids. The bioreactor was designed using computer-aided design (CAD) software and fabricated by producing negative molds through stereolithography (SLA) 3D printing, followed by silicone casting with polydimethylsiloxane (PDMS). A transparent lid enabled non-invasive monitoring of tissue morphology using optical coherence tomography (OCT). Ex vivo porcine corneas were mounted in the bioreactor using a 3D-printed ring that sealed the two silicone components, creating separate compartments for the apical and basolateral media circuits. The corneas were dynamically cultured for up to five days. Tissue morphology was evaluated through hematoxylin and eosin (HE) staining, while immunofluorescent staining was used to evaluate the expression of key epithelial markers. Wound healing was assessed using OCT imaging, enabling the observation of epithelial defect closure.

Results

Dynamic cultivation in the bioreactor preserved the multilayered corneal epithelium, as confirmed by HE staining, and enhanced the expression of epithelial markers, including N-Cadherin, CK12, CK14, and the proliferation marker Ki67. Wound healing studies showed rapid epithelial closure by day 4, while statically cultured corneas exhibited notable loss of epithelial tissue.

Conclusions

The silicone-based flow bioreactor represents a cost-effective and accessible platform for the dynamic cultivation of ex vivo porcine corneas, thereby improving tissue preservation and accelerating wound healing. The transparent design allows for real-time monitoring of tissue morphology. Furthermore, the system can be modified for the dynamic cultivation of corneal 3D in vitro models by substituting the ring with an insert membrane, enabling the application of mechanical forces and the analysis of their effects on tissue-engineered constructs.

Topic: Tissue-Specific Focus

Subtopic: Ocular tissues

Type: Poster presentation

TERMIS25_1494 Squid gladius as a marine-derived natural biomaterial for cornea tissue engineering. Jesús Chato-Astrain[1], Ingrid Garzón[1], Miguel Etayo-Escanilla[1], Juan Muñoz-Hurtado[2], Juan Pereira-Martínez[2], Juan de la Cruz Cardona[1], Ana Ionescu[1], María del Mar Pérez-Gómez[1], Carmen González-Gallardo[3], Miguel Alaminos[1]

[1]University of Granada, Spain, Instituto de Investigación Biosanitaria ibs.GRANADA, Spain, Granada, Spain; *[2]University of Granada, Spain, Granada, Spain*; *[3]Division of Ophthalmology, University Hospital San Cecilio, Instituto de Investigación Biosanitaria ibs.GRANADA, Spain, Granada, Spain*

Introduction/Objectives

Corneal tissue engineering demands biomaterials with excellent biocompatibility, biomechanical strength, and appropriate optical properties [1]. This study investigates the potential of the squid gladius (SG), a marine-derived biomaterial previously showing potential usefulness in tissue engineering [2], as a novel scaffold for cornea tissue engineering. The potential of this natural biomaterial to support recellularization with corneal cells will be determined.

Methods

SG disks were prepared and analyzed to evaluate their structural and biomechanical properties, along with their optical properties using a visible light spectrum range. Then, SG were grafted in laboratory rats, followed by histological evaluation, to determine *in vivo* biocompatibility of these biomaterials. Corneal epithelial cells were then cultured on the surface of SG biomaterials to determine the capability of these products to promote cell proliferation and differentiation.

Results

The SG displayed a homogeneous, acellular histological structure with layered lamellae and low porosity. Biomechanical testing revealed a high modulus of Young, that tended to decrease after cells were cultured on these surfaces. Optical assessments indicated high transmittance and low reflectance in SG samples. *In vivo* grafting in laboratory animals demonstrated biocompatibility, with the presence of a local initial self-limited inflammatory response without systemic adverse effects. Recellularization supported corneal epithelial attachment and viability, although differentiation of the cultured cells was not complete.

Conclusions

The squid gladius demonstrated significant potential as a novel biomaterial for cornea tissue engineering. It combines favorable intrinsic biomechanical and optical properties with no significant adverse effects once grafted *in vivo*, thus offering a promising solution derived from marine sources. Further research is needed to optimize the material for the generation of a full-thickness human cornea by tissue engineering, particularly with respect to cell differentiation and long-term *in vivo* performance.

This work was supported by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities, grants FIS PI23/00335 and ICI21/00010 (NANOULCOR). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

References:

[1] Bonato, P., and Bagno, A. (2024). Replace or Regenerate? Diverse Approaches to Biomaterials for Treating Corneal Lesions. *Biomimetics (Basel)* 9, 202

[2] Lim, T., et al. (2020). A decellularized scaffold derived from squid cranial cartilage for use in cartilage tissue engineering. *J Mater Chem B* 8, 4516–4526

Topic: Tissue-Specific Focus

Subtopic: Oral, perioral & maxillofacial tissues

Type: Poster presentation

TERMIS25_248 Biomimetic collagen-based bilayer scaffolds for effectual dentin pulp regeneration. Srinivetha Pathmanapan[1 2], Suresh Kumar Anandasadagopan[3 4]

[1]Biochemistry and Biotechnology Laboratory, Central Leather Research Institute Counsil of Scientific and Industrial Research (CSIR), Adyar, Chennai, India; *[2]Department of Leather Technology (Housed at CISIR-Central Leather Research Institute) Alagappa college of Technology, Anna university, Chennai-600020., Chennai, India*; *[3]Biochemistry and Biotechnology laboratory, Central Leather Research Institute, Council of Scientific and Industrial Research (CSIR), Adyar, Chennai, India*; *[4]Department of Leather Technology (Housed at CISIR-Central Leather Research Institute) Alagappa college of Technology, Anna university, Chennai, India*

Introduction/Objectives

Dentin-pulp complex is a unique tissue which is composed of both soft and hard tissues. Trauma and caries damage the dentin –pulp complex and it will progress to irreversible pulpitis and even tooth loss occurs if left untreated. Traditional endodontic treatments include pulpectomy and Root Canal Therapy (RCT), which involves the extirpating the damaged pulp and filling the root canal with some bioinert materials. RCT have complicities such as losing the vitality of tooth and its natural defense mechanisms against caries lesions, liable to fractures, discoloration and microleakage. At present, tissue engineering science has emerged as an appropriate choice in the field of regenerative endodontics, where, it focuses on restoring the damaged dental pulp with its own physiological function including sensibility of pulp, pulp immunity and pulp repair potential by mineralization. Thus, in our study we have fabricated and characterized the composite biphasic scaffold to be a potent biomaterial for regeneration of dentin-pulp complex.

Methods

We have fabricated the biomaterial as bilayer, where the pulp layer was fabricated with organic polymer (Collagen –I and Fibrin) as a bioactive molecule and dentin layer was fabricated using inorganic composite (Graphene oxide, nano-hydroxyapatite) which mimics the natural Extra Cellular Matrix (ECM) and microenvironment of the Dentin-pulp complex. The fabricated scaffolds were evaluated for the porous architecture, porosity, compressive strength, swelling potential, biodegradation, bio-mineralization, hemocompatibility, biocompatibility and differentiation. The biocompatibility of scaffolds was evaluated by cell viability and cell adhesion assays. Then the osteogenic and chondrogenic differentiation potential of the biphasic scaffold were analyzed at both cellular and molecular level by Alkaline phosphatase assay ALP, Alizarin red staining, Glycosaminoglycans (GAG) analysis and gene expression studies.

Results

Bilayer scaffolds were subjected to structural and physicochemical characterization which exhibits an integrated layer with suitable pore sizes, high porosity and optimum mechanical strength. average pore size in the chondral layer is 100–200 μm, and in the subchondral layer, it is 250–350 μm. The fabricated biomaterial is biocompatible with enhanced mineralization and the bilayer scaffold exhibits 53.15% degradation after 28 days, which correlates with the natural bone resorption rate. Alizarin red staining and Alican Blue staining results showed the osteogenic and chondrogenic differentiation potential of the

fabricated scaffolds. The Glycosamino glycan estimation proved that fabricated bilayer scaffold enhances chondrogenesis and neo-ECM secretion. The engineered biomaterial could also increase the upregulation of odontogenic and chondrogenic gene expression.

Conclusions

Thus, we have fabricated a biphasic scaffold as a cell–free biomaterial that holds potent advantages in promoting tissue repair with economic and low risk. The findings suggest that the fabricated bilayer scaffold has an effectual osteoinductive and chondroinductive potential which will be a potent biomaterial for complete restoration of Dentin pulp regeneration.

Topic: Tissue-Specific Focus

Subtopic: Oral, perioral & maxillofacial tissues

Type: Poster presentation

TERMIS25_433 Necroptosis inhibitor ameliorate the disease progression of medication-related osteonecrosis of the jaw: a preclinical study using 18F-fluorodeoxy glucose PET/CT. Yemi Kim[1], Byung Seok Moon[2], Seo Young Kang[2], Ji-Young Kim[2], Hai-Jeon Yoon[2], Daehee Kim[3], Sung June Jang[4], Bomsahn Kim[2]

[1]Department of Conservative Dentistry, College of Medicine, Ewha Womans University, Seoul, Korea, Rep. of South; *[2]Department of Nuclear Medicine,College of Medicine, Ewha Womans University, Seoul, Korea, Rep. of South*; *[3]Department of Emergency Medicine, Incheon St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, Rep. of South*; *[4]Department of Nuclear Medicine, Sheikh Khalifa Specialty Hospital, Ras Al Khaimah, United Arab Emirates*

Introduction/Objectives

Medication related osteonecrosis of the jaw (MRONJ) is an irreversible adverse complication related with anti-resorptive medications. The purpose of this study was to evaluate the therapeutic effect of necroptosis inhibitor using 18F-FDG PET/CT in rat models treated with zoledronic acid (ZA) followed by tooth extraction.

Methods

A total of fourteen rats were divided randomly into two groups; group 1, rat treated with ZA; Group 2, rat treated together with ZA and necroptosis inhibitor. followed by tooth extraction. They underwent examinations with 18F-FDG PET/CT at 4 weeks after tooth extraction. Radiological evaluation of all groups were compared to assess the effects of therapeutic medication. Rats were then sacrified to evaluate the histological features of MRONJ.

Results

18F-FDG PET/CT performed at 4 weeks after tooth extraction showed more improved glucose metabolism at the extraction site in ZA with necroptosis inhibitor group compared with that in the ZA only group (maxSUV: 1.76 vs. 2.47; $p < 0.006$). ZA with necroptosis inhibitor group showed low level of MLKL and improved activity of RUNX2 and IL-6 than those of ZA group.

In comparison with ZA group, ZA with necroptosis inhibitor group had more improved state of empty lacuna(2.12 vs. 7.05 %) and repaired tendency of necrotic bone formation(1 vs 5.76 %).

Conclusions

Our findings suggest that the appilcation of necroptosis inhibitor could be used as a useful treatment against a possible risk of MRONJ.

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (RS-2024-00449684, NRF-2022R1A2C1004175 and RS-2024-00439928).

Topic: Submit to SYMPOSIUM

Subtopic: Tissue Engineering and Biofabrication for Regenerative Dentistry

Type: Poster presentation

TERMIS25_1137 Synergetic effects of injectable platelet-rich fibrin and commercially available tissue engineering materials on growth factor release and osteoclast activity.

Lauma Ievina[1 2], Elena Della Bella[2 3], Lana Micko[2 4 5], Ilze Salma[2 4 5], Alexander Sieberath[6], Arita Dubnika[1 2]

[1]Institute of Biomaterials and Bioengineering, Riga Technical University, Riga, Latvia; *[2]Baltic Biomaterials Centre of Excellence, Riga, Latvia*; *[3]AO Research Institute Davos, Davos Platz, Switzerland*; *[4]Institute of Stomatology, Riga Stradins University, Riga, Latvia*; *[5]Department of Oral and Maxillofacial Surgery, Riga Stradins University, Riga, Latvia*; *[6]Department of Experimental Surgery- University Hospital Knappschaftskrankenhaus Bochum, Bochum, Germany*

Introduction/Objectives

Platelet-rich fibrin (PRF) is a protein matrix with growth factors and immune cells extracted from venous blood via centrifugation. Studies have demonstrated its effectiveness as a biocompatible biomaterial for bone and soft tissue regeneration in dental surgeries, with easy modification for composite preparation. However, the effectiveness of combining PRF with other commercially available biomaterials remains inconclusive, possibly due to the interactions between PRF and these biomaterials. While some studies have explored combining PRF with similar materials, like different types of calcium phosphate scaffolds, there is a lack of research comparing the interactions between injectable-PRF (i-PRF) and various sources of materials. This study aims to compare the duration of growth factor release from i-PRF when combined with different biomaterials and to evaluate how these combinations influence osteoclast differentiation and activity.

Methods

i-PRF was isolated from donor blood by centrifugation (5 min, 700 rpm) and combined with collagen (Collacone, Biotiss), bone substitute/collagen (Bio-Oss, Geistlich), and hydroxyapatite (HA)/β-tricalcium phosphate (β-TCP) (CeraForm, Teknimed) scaffolds by impregnation. The cytokine release from these composites was measured over 10 days in α-MEM. The effect of PRF-composite conditioned media (with the addition of RANKL and M-CSF) on osteoclast differentiation and activity was tested on both regular cell culture plastic plates and plates coated with simulated body fluid (SBF) and type I collagen using a patented coating technique to achieve bone-like coating on plates. RT-qPCR analysis was conducted on day 10 to measure TRAP, cathepsin K, RANK, matrix metallopeptidase 9, and carbonic anhydrase 2 gene expression in osteoclasts grown on regular 96-well plates. Osteoclast activity was assessed on bone-like calcium phosphate-coated plates by measuring coating resorption and staining cells with actin, DAPI, and TRAP.

Results

i-PRF combined with biomaterial scaffolds exhibited similar endothelial growth factor, platelet-derived growth factor, and transforming growth factor release intensity to i-PRF alone. Collagen-containing scaffolds induced strong pro-inflammatory cytokine (Interleukin-6 and interleukin-8) release from i-PRF, while HA/β-TCP scaffolds and i-PRF alone did not. The presence of i-PRF enhanced osteoclast calcium resorption from day 3 to day 8 and TRAP stain intensity on the coated plates compared to materials alone. However, gene expression between materials with or without i-PRF was not significantly different by day 10.

Conclusions

Collagen-containing biomaterials cause pro-inflammatory cytokine release from i-PRF, an effect less evident in CaP materials. i-PRF could enhance early osteogenic differentiation and osteoblast activity with or without the addition of collagen or CaP materials.

Topic: Submit to SYMPOSIUM

Subtopic: Biophysical Therapies for Tissue Regeneration

Type: Poster presentation

TERMIS25_141 Improvement of barrier function in three-dimensional skin substitutes by photobiomodulation during cultivation. Yasuyuki Tsunoi[1], Hiromi Miyazaki[1], Mitsuru Akashi[2], Satoko Kawauchi[1]

[1]National Defense Medical College Research Institute, Tokorozawa, Japan; *[2]Osaka University, Suita, Japan*

Introduction/Objectives

In mass disasters and terrorism, many victims may suffer from extensive skin damage, requiring a large number of skin grafts for transplantation. In such cases, allogeneic skin grafts are useful, but they are not always available because of the limited number of donors. Skin substitutes, such as artificial skins and cultured skins, can also be used. However, current commercially available skin substitutes lack vasculature, which cause delayed perfusion and hence easy infection after transplantation. Considering these facts, we are currently developing a three-dimensional (3D) cultured skin model containing vascular networks. However, we faced the problem of decreased tissue quality, such as deteriorated barrier function, owing to inefficient nutrient transport from the surrounding culture medium to the thick 3D skin. To solve this problem, we applied photobiomodulation (PBM), which can activate the electron transport chain of mitochondria and thus cells and tissues by illumination with low-power light.

Methods

Human dermal fibroblasts (2 x 10^6 cells) were coated with fibronectin and gelatin to promote cell-cell adhesion, mixed with human umbilical vein endothelial cells (2 x 10^4 cells), and seeded onto a 24-well culture insert to form a dermis. Human epidermal keratinocytes (6 x 10^5 cells) were then seeded onto the dermis to form an epidermis. Thereafter, the epidermis was lifted to the air-liquid interface to drive differentiation. From this timepoint, PBM at 823 nm, which was shown to be effective in accelerating adenosine triphosphate (ATP) production in 3D cultured skin in our previous study, was applied daily for five consecutive days; the 3D skins were illuminated with an 823-nm light-emitting diode (LED) array (100 J/cm^2) from the dermal side. Twenty-four hours after the final PBM application, the expression level of filaggrin, a terminal differentiation marker of epidermal keratinocytes, in the 3D skin was evaluated by immunohistochemical staining and enzyme-linked immunosorbent assay because skin barrier function is acquired as the differentiation of the epidermis is promoted. In addition, the transepithelial electrical resistance (TEER) of the 3D skin was measured to assess barrier function. The results of these measurements were statistically compared with those of 3D skins without PBM application (control cultures) using two-tailed t test.

Results

The expression level of filaggrin in the epidermis of the PBM group was 15% higher than that of the control group, and there was a significant difference ($p<0.05$) between these groups. The electron transfer reaction produces reactive oxygen species (ROSs), as well as ATP, and ROSs are known to mediate the differentiation of epidermal keratinocytes. This may be a mechanism by which PBM promoted the epidermal differentiation of the 3D skins. The TEER value in the PBM group was 67% higher than that in the control group, and there was a significant difference ($p<0.01$) between these groups. Infection is a major cause of graft failure in skin transplantations. Therefore, the application of PBM is crucial for the cultivation of 3D skin substitutes with excellent barrier properties.

Conclusions

PBM promoted the epidermal differentiation and improved the barrier function of the 3D skin during cultivation.

Topic: Tissue-Specific Focus

Subtopic: Skin, allografts

Type: Poster presentation

TERMIS25_1160 Biologically Relevant Support for Primary Human Keratinocytes, Melanocytes and Fibroblasts, under Xenofree- and Chemically Defined Conditions. Boris Eleuteri[1], Malin Kele[1], Therése Kallur[2]

[1]*BioLamina AB, Sundbyberg, Sweden*; [2]*BioLamina Inc, Cambridge, United States*

Introduction/Objectives

Autologous skin cultures are one of the first in vitro expanded tissue types used in cell therapies. Pioneering research used expanding human keratinocytes on murine feeder cells and bovine serum enriched medium. However, the use of animal components is the major factor restraining the treatment of wounds to only the severest cases.

Protocols for pluripotent-based cell therapies have transferred from animal-components to xenofree-and defined settings by the development of recombinant laminin proteins as coating substrate. Laminins are an extracellular matrix protein family highly expressed within the basement membrane (BM) in all epithelial tissues. BM is a vital part of the stem cell niche supporting the basal keratinocytes in tissue homeostasis and in wound healing. The BM within the skin contains multiple laminin isoforms, where laminin-332, -331, laminin-511, -521, laminin-421 and -411 are the major components. Although the distinct role of the different laminin isoforms is still largely unknown, the essential role in maintaining healthy skin is demonstrated in Epidermolysis bullosa, a severe genetic skin disease caused by a mutation in laminin 332, leading to fragile skin and chronic wounds.

Methods

We use recombinantly produced laminins, Biolaminins, as cell culture substrates for human primary keratinocytes, melanocytes, and fibroblasts. They can provide a biologically relevant support to the major cell types of the skin also from adult donors.

Results

We demonstrate that the effect is laminin isoform dependent and provides superior support to the proliferative cells within the skin, also from aged donors, compared to current methods. For example, the use of Biolaminin-521 in expanding adult primary keratinocytes resulted in 14 cumulative doublings compared to 8 with standard conditions after 30 days in cultures. Furthermore, the use of Biolaminins provides a better support for survival- and growth of freshly isolated skin cells resulting in a 4 times higher cellular yield.

Conclusions

In conclusion, this allows for a new generation of safe- and efficient *in vitro* expansion of human keratinocytes, melanocytes, and fibroblasts, under completely xenofree and chemically defined replacing the current, undefined, and often serum-based alternatives. We believe that this method of expanding adult human skin cells can be applicable to cell-based wound care, potentially also expanding the clinical use.

Topic: Tissue-Specific Focus

Subtopic: Skin, allografts

Type: Poster presentation

TERMIS25_1495 Generation of multicellular human skin equivalents to study toxicological injuries. Catalina Gaviria[1], Lalitha Karchalla[1], Anthony Atala[1], Patrick McNutt[1]

[1]*Wake Forest Institute for Regenerative Medicine, Winston-Salem, United States*

Introduction/Objectives

Biological and chemical weapons represent a global concern due to their significant impact on morbidity and mortality. Among these, warfare agents and poxviruses are considered critical potential threats because of their ability to cause severe systemic disease, the lack of effective therapeutic treatments and the limited knowledge on mechanisms of toxicity. A major challenge in studying skin pathophysiology is the lack of sophisticated human skin models. Most existing models consist only of keratinocytes and fibroblasts and

thus neglect the influence of endothelial and adipose tissues on skin homeostasis and pathogenesis. Since skin function and immune defense rely on interactions between various cell types, more physiologically relevant systems that better mimic the complexity of human skin are highly desirable. To address this limitation, we developed a multicellular human skin equivalent (HSE) that accurately replicates human skin biology and can be used to study toxicological injuries.

Methods

We generated a human skin equivalent (HSE) consisting of a stratified epidermis with keratinocytes and subepithelial fibrin matrix containing fibroblasts, adipocytes, and endothelial cells. The HSE was validated in terms of structure, protein expression, and functionality of the epidermal barrier. To demonstrate their utility in skin toxicology research, HSEs were exposed to vesicant agents and poxviruses, including vaccinia, cowpox and monkeypox. We then analyzed histological and genotoxic damage along with protein and transcriptional changes following exposure.

Results

HSEs faithfully recapitulated the human skin structure and function, expressing markers of differentiated epithelium. Endothelial cells spontaneously formed capillary-like structures within the fibrin gel. We confirmed the susceptibility of the HSEs to vesicants and viral infection through immunohistochemical imaging and observed cytopathic effects such as blistering and DNA damage. We also identified key signaling pathways involved in virus recognition and antiviral responses. Monkeypox, vaccinia, and, to a lesser extent, cowpox viruses were found to downregulate interferon signaling and pathogen recognition pathways while upregulating anti-inflammatory cytokines, mimicking typical orthopoxvirus immune evasion mechanisms.

Conclusions

This multicellular HSE exhibits strong potential as a platform to studying skin toxicology. By incorporating multiple cell types, it provides a more authentic representation of human skin responses to various injuries, offering additional targets for therapeutic development. Because HSEs do not include immune cells, we are recapitulating innate signaling pathways that initiate defense mechanisms, which cannot be studied in vivo. It is anticipated that HSEs will make substantial contributions to the fundamental understanding and treatment of toxicological insults in the context of phenotypically mature, human skin tissues.

Topic: Submit to SYMPOSIUM

Subtopic: Tendon regeneration: lights and shades

Type: Poster presentation

TERMIS25_195 Enrichment of Bioinks with Growth Factor-Loaded Nanoparticles: A Novel Approach to Improve Scaffolds Efficacy in Tendon Regenerative Medicine. Jorge Ordoyo-Pascual[1 2 3], Sandra Ruiz-Alonso[1 2 3], Idoia Gallego[1 2 3], Laura Saenz-del-Burgo, Jose Luis Pedraz

[1]NanoBioCel Research Group, Laboratory of Pharmacy and Pharmaceutical Technology. Department of Pharmacy and Food Science, Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Paseo de la Universidad 7, 01009, Vitoria-Gasteiz, Spain; *[2]Bioaraba, NanoBioCel Research Group, 01009, Vitoria-Gasteiz, Spain*; *[3]Networking Research Centre of Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Institute of Health Carlos III, 28029, Madrid, Spain*

Introduction/Objectives

With the increasing aging of the global population, the structural integrity and functionality of tendons deteriorate, presenting a challenge to healthcare systems. In response, regenerative medicine and tissue engineering have emerged as viable alternatives through bioinks printing. However, it is sometimes necessary to enrich these bioinks with biologically active molecules, such as growth factors, in order to

induce effects like angiogenesis and cell growth. However, these molecules are sensitive to degradation by physical and chemical agents, making their encapsulation in nanoparticles (NPs) essential.

Methods

For this purpose, nanostructured lipid carriers (NLCs) loaded with Vascular Endothelial Growth Factor 165 (VEGF165) and Platelet-Derived Growth Factor-BB (PDGF-BB) were incorporated into a bioink containing nanocellulose, alginate and hyaluronic acid. The bioink and nanoparticle suspensions were prepared in separate syringes and subsequently homogenized by transferring their contents to each other through a connector. The resulting bioink, enriched with NLCs, was optimized by evaluating its rheological properties and performing a printability study to determine the optimal printing conditions. Finally, the study of the release of growth factors was performed.

Results

The incorporation of nanoparticles into the bioinks did not result in significant changes to the viscoelastic properties of the hydrogel. However, viscosity analysis indicated a different behavior following the addition of NLCs, with viscosity increasing as higher quantities of nanoparticles were incorporated. This effect was particularly pronounced at lower shear rates. Similarly, assessments of stiffness, work and maximum force indicated a consistent trend: greater NP concentrations corresponded to higher values in these parameters. Subsequently, the printability of the bioink was evaluated without NLCs and with NLCs at concentrations of 0.6 mg/mL and 3.4 mg/mL. Both the nanoparticle-free hydrogel and the hydrogel containing 0.6 mg/mL of NLCs were printable at 30 kPa, whereas the bioink with 3.4 mg/mL of nanoparticles required a higher pressure of 35 kPa. Finally, the release profile of growth factors from the NLCs was evaluated, both within and outside the scaffold. It was observed that the release of the growth factors from NPs was quick, following a “burst release” profile, with the majority being liberated within the first 10 hours. In contrast, the release of factors from NPs within the scaffolds showed a different profile. A more sustained release over time was observed, with the greatest amount of factor being released by day 8.

Conclusions

In conclusion, the incorporation of growth factor-loaded NLCs induces slight modification in the rheological properties of the bioink, requiring an increase in printing pressure. Additionally, a much more gradual and sustained release profile from the scaffold is achieved. This approach allows both the cells within the scaffold and those in the tendon tissue to proliferate and induce vascularization, thereby increasing the healing process. Consequently, the use of VEGF165 and PDGF-BB-loaded NPs for bioink enrichment represents a promising strategy for the treatment of tendon pathologies.

Topic: Tissue-Specific Focus

Subtopic: Tendons & ligaments

Type: Poster presentation

TERMIS25_586 A trace element-deficient diet modulates the structure and gene expression of murine Achilles tendons.

Manuela Thierbach[1], Kristina Lossow[2], Anna Kipp[2], Britt Wildemann[1]

[1]*Experimental Trauma Surgery, Jena, Germany*; [2]*Institute of Nutritional Sciences, Jena, Germany*

Introduction/Objectives

The relationship between diet and musculoskeletal tissue properties is of increasing interest. A recent meta-analysis on the effect of nutrition on tendon tissue found only 19 studies that met the inclusion criteria (Hijlkema et al. 2022, PMID: 35937777). The aim of this study was therefore to investigate the influence of a trace element-deficient diet on mice Achilles tendons.

Methods

C57BL/6JRj mice (males and females) were fed trace element-deficient (insufficient supply of copper, zinc and selenium, TED animals) or adequate (control animals) diets from week 11th after birth; n=17 per

group (Approval: FSU-20-003). After 8 weeks, the Achilles tendons were harvested (both sides) and the tibial lengths were determined. Tendon histology: number of tenocytes (cell nucleus count), intercollagen fiber spaces and organization of collagen fibers. qPCR: genes for extracellular matrix proteins, tenocyte markers, matrix metalloproteases, interleukins, Wnt signaling cascade. Normalized expression was calculated according to Simon P., Bioinformatics, 2003. Statistics: Mann-Whitney U test, SPSS 27.

Results

The tibiae of the TED animals were significantly shorter than those of the control animals. There was a tendency towards higher cellularity in the Achilles tendons of the TED animals compared to the control animals. A disorganization of the collagen fibers could not be observed. However, the intercollagen fiber spaces in the Achilles tendons of the TED animals were significantly larger than in the control animals.

In the Achilles tendons of TED animals, collagen1a1 (*Col1a1*), elastin (*Eln*) and the tenocyte marker tenomodulin (*Tnmd*) were expressed at significantly lower levels than in control animals. The proteoglycans biglycan (*Bgn*) and versican (*Vcan*), the Wnt signaling cascade protein ß-catenin (*Ctnnb1*) and the vascular endothelial growth factor D (*VegfD*) were significantly higher expressed in the Achilles tendons of TED animals.

Conclusions

Copper, zinc and selenium deficiency alters mouse Achilles tendons both histologically and on the molecular level. The larger intercollagen fiber spaces, the higher cellularity, the lower expression of the main structural proteins *Col1a1* and *Eln* and the tenocyte marker *Tnmd* as well as the higher expression of the proteoglycans *Bgn* and *Vcan* in the TED tendons could indicate impaired property of the tendons, e.g. with regard to their compactness/strength/elasticity. Furthermore, the Achilles tendons of males and females seem to react differently to the trace element deficiency. The increased expression of *Ctnnb1* and *VegfD* could indicate an influence on the Wnt signaling pathway, e.g. due to selenium deficiency. This connection has already been described for the colon (e.g. Kipp et al., 2009, PMID: 19810021) and Wnt signaling can influence tendon markers in rat tendon derived cells (Kishimoto et al., 2017, PMID: 28750046). These data form the basis for further research into the influence of trace elements on tendon integrity.

Topic: Tissue-Specific Focus

Subtopic: Tendons & ligaments

Type: Poster presentation

TERMIS25_779 Functionalized double-geometry meltelectrowritten scaffolds for bone to tendon interface engineering. Camilla Mussoni[1], Taufiq Ahmad[1]

[1]*University of Würzburg, Würzburg, Germany*

Introduction/Objectives

Motion is produced by muscle contraction and for it to translate into movements of limbs, muscles need connection the bone and this is the very important function of tendons. Mismanaged movements can cause overstraining and tendon injuries, occurring very commonly, from simple inflammation to proper disruption. Repairs of the more severe injuries through regeneration is a well-known challenge in medicine/tissue engineering to answer to a major societal issue. The advent of additive manufacturing technologies brought forward new possibilities to engineer scaffolds to support tissue formation by designing geometries and structures replicating the natural. The aim of this study is to fabricate through melt electro writing (MEW) the bone-tendon interface with dual geometry – stiffer for the bone and more elastic for the tendon - and functionality – a mineralized environment for the bone and collagen rich for the tendon.

Methods

The scaffolds are fabricated by MEW of Polycaprolactone (PCL) with a custom-built device in one print. For the bone side a simple box geometry has been chosen while for the tendon a sinusoidal pattern to mimic

the natural structures of the tissue. To offer a more hydrophilic cell-friendly surface the scaffolds are coated in Polydopamine as agent for immobilization of Gelatine Type B. Furthermore, the bone half of the scaffold is mineralized with a Simulated Body Fluid solution in vacuum for 24 hours. Mechanical tests are performed to assess the properties of the two different areas. Adipose derived Stem Cells (ASC) are seeded on the scaffolds and cultivated for up to 5 days.

Results

The scaffolds present a dual geometry of squared boxes and sinusoidal waves with a repetitive unit of 0.5 mm for 20 layers, with a resulting height of 0.7 mm. Raman spectroscopy and micro-BCA confirm a successful functionalization through dip coating and mineralization. Cells show good survival and attach on the scaffold till full colonization; within 5 days they spread to fill the gap between the fibers. Mechanical tests, both tensile and cyclic, show a viscoelastic behavior of the tendon half with a typical J shape curve due to the initial distention of the fibers before the full load is applied and plastic deformation occurs. Maximum stress measured is 4 kPa and Young Modulus is calculated to be 20 kPa.

Conclusions

In this study we propose meltelectrowriting as technique to produce bone-tendon interface scaffolds for tissue engineering. We designed a fitting geometry to recapitulate the tissue properties and proved its properties through evaluation of the mechanical properties. Furthermore, the scaffold is functionalized through two different coating for each of the tissues. Future studies will focus on ASC cell's differentiation.

Topic: Tissue-Specific Focus

Subtopic: Tendons & ligaments

Type: Poster presentation

TERMIS25_988 Characterizing the tendon healing process in an acute rat model of Achilles defect. Yang XU[1], Yuxin Wang[2], Yuanhao Zhang[2], Hoi Pan Harry Cheung[2], Dan Wang[2]

[1]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, HongKong, China*; [2]*The Chinese University of Hong Kong, HongKong, China*

Introduction/Objectives

Tendon healing is a complex, multi-phase process involving structural, mechanical, and compositional changes, and the highly aligned collagen structure is believed to be crucial for restoring tendon functionally mechanical properties. However, a lack of understanding regarding the cause of the misalignment of the neonatal tendon has hindered efforts to effectively reconstruct aligned tendon structures during the healing process. Traditional assessment methods, which largely focus on macroscale tensile strength, collagen alignment, and off-target tissue formation, do not fully capture the intricacies of tendon repair. This study seeks to address these limitations by assessing structural alignment, mechanical properties, and composition at both the tissue (macroscale) and micro-nanoscale levels at multiple time points.

Methods

An acute Achilles tendon defect was created in Sprague-Dawley rats with a 2-mm full-thickness defect. Tendons were immediately repaired, and samples collected at 1-, 2-, 4-, and 8-weeks post-surgery. Tendon integrity and morphology were assessed using ultrasound and gross examination. Structural alignment was evaluated via histological staining and scanning electron microscopy (SEM). Mechanical properties were assessed through tensile testing and atomic force microscopy (AFM), while ECM composition was analysed using histological staining and Raman spectroscopy. Statistical analysis was conducted using GraphPad Prism 9, with $p < 0.05$ considered significant.

Results

Ultrasound and gross examination revealed tendon swelling and elongation in defect groups at 4 and 8 weeks. Histological staining showed less organized collagen fibers and decreased alignment in these groups at all time points. SEM indicated reduced collagen fibril alignment and smaller diameters at the micro-nanoscale. These changes were associated with reduced tissue stiffness, increased chondrification, calcification, and ECM alterations. Tensile testing showed lower mechanical strength, with AFM revealing reduced fibril stiffness. Elevated chondrification and calcification were noted at 4 and 8 weeks, with scar tissue formation indicating insufficient healing. This study highlights trends in structural disorganization, decreased mechanical strength, and compositional changes at multiple scales, previously unexplored. Notably, increased collagen diameter did not prevent decreased tissue strength, challenging previous assumptions.

Conclusions

This study convinced that the misalignment of the tendon tissue and the decreased tensile strength occur alongside lower tissue stiffness, increased cell differentiation, and altered tissue composition. Further correlation analysis will be produced to identify properties which are most related to misalignment and dysfunction at various healing stages. Understanding multiscale tendon property changes during healing can inform advanced therapeutic approaches and reliable assessment methods targeting key changes at critical time points.

Topic: Tissue-Specific Focus

Subtopic: Tendons & ligaments

Type: Poster presentation

TERMIS25_1351 TENDON PROGENITOR STEM CELLS: A NEW TOOL FOR INVESTIGATING TENDON REGENERATIVE EVENTS. Erwin Pavel Lamparelli[1], Maria Camilla Ciardulli[2], Nicola Maffulli[3], Giovanna Della Porta[4]

[1]Translational NanoMedicine Laboratory, Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi, SALERNO, Italy; *[2]Department of Biomaterials and Biomedical Technology, University Medical Center Groningen, Groningen, Netherlands*; *[3]Department of Trauma and Orthopaedics, Faculty of Medicine and Psychology, Sant' Andrea Hospital, ROME Sapienza University, Rome, Italy*; *[4]Translational NanoMedicine Laboratory, Department of Medicine, Surgery and Dentistry, University of Salerno, Research Centre for Biomaterials BIONAM, Università di Salerno, Fisciano, SA, Italy, Baronissi, SALERNO, Italy*

Introduction/Objectives

Tendon progenitor stem cells (TPSCs) represent a promising avenue for studying and understanding tendon regenerative processes. These multipotent cells, derived from tendon tissue, possess the ability to differentiate into tenocytes and other mesenchymal lineages, making them valuable for exploring the cellular and molecular mechanisms underlying tendon repair and regeneration. By serving as an in vitro model, human TPSCs allow to investigate the dynamics of extracellular matrix production, growth factor signaling, and cellular interactions critical for tendon healing. Furthermore, their role in pathological conditions and response to therapeutic interventions can be studied, offering insights into novel regenerative strategies

Methods

Human Tendon Progenitor Stem Cells (hTPSCs) were successfully isolated from both healthy and pathological tendon surgery explants (Review Board protocol no. 151, approved on 29.10.2020). These cells were cultured in both two-dimensional (2D) monolayer systems and three-dimensional (3D) environments, including spheroids and bioplotted hydrogels (density 2 and 1^6 cells/mL) such as collagen Methacrylate (ColMA) or gelatin Methacrylate (GelMA) under both static and dynamic (perfusion) conditions to simulate physiological and pathological microenvironments.

Results

Proposed data offers an overview about Human Tendon Progenitor Stem Cells (hTPSCs) (healthy and pathological ones) cultures in both 2D and 3D environment with the addition of different Transforming Growth Factors (TGFs) such as Growth Differentiation Factor 5 (GDF-5, 100 ng/mL) or TGF-β1 (20 ng/mL), or BMP-2, (20 ng/mL), and alternatively, specific lipid nanoparticles (LNP) carrying hormones and/or proteins, tailored to evaluate their regenerative potential or therapeutically activity. Across these diverse culture setups, hTPSCs exhibited remarkable adaptability and functional capacity. They responded effectively to the biochemical and biomechanical stimuli, expressing specific genotype and offering valuable insights into tendon biology and the interplay between cellular behavior and the surrounding environment. These findings underscore the utility of hTPSCs in uncovering mechanisms of tendon regeneration and the development of targeted therapeutic strategies.

Conclusions

Human Tendon Progenitor Stem Cells (hTPSCs) serve as a pivotal resource for advancing tissue engineering and innovating tools for *in-vitro* model of tendon tissue engineering. Their unique regenerative potential and responsiveness to diverse environmental cues position them as a cornerstone for improving outcomes in tendon repair and regenerative medicine.

Topic: Tissue-Specific Focus

Subtopic: Urinary system

Type: Poster presentation

TERMIS25_675 Scalable manufacturing of human smooth muscle cells for bladder tissue engineering. Teresa Franchi-Mendes, Marília Silva[1 2], Maria Catarina Carreira[1 2], Ana Luísa Cartaxo[1 2], Paulo Vale[3], Panagiotis Karakaidos[4], Apostolos Klinakis[4], Ana Fernandes-Platzgummer[1 2], Cláudia Lobato Da Silva[1 2]

[1]Department of Bioengineering and iBB - Institute for Bioengineering and Biosciences at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; *[2]Associate Laboratory i4HB - Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal*; *[3]Urology Department, Hospital CUF Descobertas, Lisboa, Portugal, Lisbon, Portugal*; *[4]Biomedical Research Foundation of the Academy of Athens, Athens, Greece, Athens, Greece*

Introduction/Objectives

Tissue engineering strategies hold a great promise as an innovative therapeutic strategy for bladder reconstruction/regeneration, which are required due to several disorders and complications associated to the urinary tract. In what concerns organ functionality, smooth muscle cells (SMC) are essential players for proper contraction and relaxation of the bladder. The demand for high numbers of SMC towards clinical applications requires the need for cost-effective culture protocols. Moreover, alternatives to the commonly employed fetal bovine serum (FBS) supplementation are required in order to facilitate clinical translation for tissue engineering purposes.

This work aimed at developing a xeno(geneic)-free cell manufacturing process, from isolation to expansion, for human bladder-derived SMC.

Methods

A gamma-irradiated human platelet lysate (HPL) supplement, able to comply with Good Manufacturing Practices (GMP), was used herein to develop a xeno(geneic)-free process for human bladder-derived SMC manufacturing. Cells were isolated from biopsies using an explant-based technique, followed by ex vivo expansion under static and dynamic conditions.

Results

Target cells were isolated from donor samples aged 58-80 years old. For cell identity, detection of typical SMC markers (α-SMA, desmin, caldesmon and SM22-α) by immunofluorescence and flow cytometry was

performed. A close-to-function assay readout based on gel contractility was also used for phenotype validation. Cell culture was successfully scaled-up to dynamic and scalable systems consisting of spinner flasks combined with plastic microcarriers coated with HPL. Upon effective cell growth for 7 days on spinner flasks, assessed by quantitative and qualitative methods, cell identity was validated and compatible to a SMC phenotype.

Conclusions

Overall, we established a xeno-free, GMP compliant, process for human bladder SMC manufacturing, and demonstrated its scalability with maintenance of cell identity.

Topic: Tissue-Specific Focus

Subtopic: Womens health

Type: Poster presentation

TERMIS25_752 Tuning ECM composition and stiffness in a peritoneal 3D in vitro model. Sema Demir[1 2], Hui-Yu Liu[3], Martin Weiss[2 4], André Koch[5], Sara Brucker[4], Bernhard Krämer[4], Katja Schenke-Layland[1 2], Peter Loskill[2 3], Julia Marzi[1 2]

[1]Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Faculty of Medicine, Eberhard Karls University Tübingen, Tübingen, Germany; *[2]NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*; *[3]Institute of Biomedical Engineering, Department for Microphysiological Systems, Faculty of Medicine, Eberhard Karls University Tübingen, Tübingen, Germany*; *[4]Department of Women's Health, University of Tübingen, Tübingen, Germany*; *[5]Research Institute for Women's Health, University of Tübingen, Tübingen, Germany*

Introduction/Objectives

Endometriosis is a disease characterized by endometrial tissue growing outside of the uterus. While its pathogenesis is not fully understood yet, one hypothesis proposes the attachment of ectopic endometrial cells to the peritoneal wall after retrograde menstruation. However, retrograde menstruation does not always necessarily lead to endometriosis, hence other mechanisms must be involved.[1]

To unravel potential mechanisms of endometriotic pathogenesis, our aim is to facilitate observations on patient-derived endometrial cells in a peritoneal environment using a microfluidic organ-on-chip platform. To achieve this, we will combine patient-derived peritoneal cells, endometrial organoids and tuneable synthetic hydrogels and thereby enable the simulation of a (patho-)physiological environment *in vitro*. By modulating characteristics of the hydrogel, the invasiveness of endometriotic cells can be studied in different peritoneal environments. Thus, the patient-specific susceptibility of a peritoneum to support growth of endometriotic lesions is explored. In this work the influence of peritoneal stiffness and its extracellular matrix (ECM) composition on cell growth and invasiveness is investigated on a microfluidic chip device.

Methods

Endometrial cells and endometriotic microtissues were cultured in different synthetic hydrogels. Utilising a range of crosslinkers and polymers as well as altering their concentrations modified the hydrogel stiffness. Additionally, the gel composition was modified by adding different ECM proteins like collagens and laminin. The embedded microtissue was placed inside a microfluidic device facilitating regular media exchange. Changes in cell morphology and behaviour were examined and evaluated via microscopy. Confocal laser scanning microscopy enabled recording of z-stacks to observe the cells in a 3D environment. Cell viability was assessed via fluorescence assays. Cell morphology as well as their production of different ECM proteins was observed by immunofluorescence staining and subsequent microscopy.

Results

Changing the ECM protein composition of the synthetic hydrogels lead to varying gel stiffness which can be used to mimic different physiological and pathological conditions in endometriotic gland formation and

fibrotic remodeling. Moreover, stiffness- and composition-dependent influences on growth, morphology and viability of the cells and microtissues were observed. Adapting the cellular microenvironment was further demonstrated to have an impact on cell-based ECM production.

Conclusions

The attainability of simulating varying ECM stiffnesses according to the wide range of peritoneal stiffnesses in female bodies by using synthetic hydrogels on microfluidic devices is shown. Differences between endometrial cell morphology and behaviour in addition to detectable changes in ECM production hint at the bigger role of peritoneal stiffness in the formation of endometriosis. The results give insight into the diverse invasiveness of endometriosis and its pathogenesis in peritoneum dependent on the peritoneal ECM composition and stiffness.

Acknowledgements

This work was supported by the German Federal Ministry of Education and Research (ENDO-RELIEF, 01EJ2403B).

[1] V. J. Young, J. K. Brown, P. T. K. Saunders, and A. W. Horne, ‘The role of the peritoneum in the pathogenesis of endometriosis’, *Human Reproduction Update*, vol. 19, no. 5, pp. 558–569, Sep. 2013.

Topic: Tissue-Specific Focus

Subtopic: Womens health

Type: Poster presentation

TERMIS25_1207 3D fallopian-tube-on-a-chip model for investigating tubal fibrosis induced by Chlamydia trachomatis. Milena Ślęczkowska[1], Jaehyeon Kim[1], Hanna Marti[2], Nicole Borel[2], Servaas Morré[3 4 5], Paul Wieringa[1]

[1]Complex Tissue Regeneration Department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands; *[2]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland*; *[3]Dutch Chlamydia trachomatis Reference Laboratory, Department of Medical Microbiology, Infectious Diseases and Infection Prevention, Care and Public Health Research Institute (CAPHRI), Maastricht University Medical Centre (MUMC+), Maastricht, Netherlands*; *[4]Institute of Public Health Genomics, Dept of Genetics and Cell Biology, FHML, GROW Research Institute for Oncology and Reproduction, Maastricht University, Maastricht, Netherlands*; *[5]Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, UP, India*

Introduction/Objectives

Chlamydia trachomatis (Ct) is the most common sexually transmitted bacterium worldwide. It primarily infects epithelial cells of the reproductive tract leading to inflammation resulting in symptoms like painful micturition, pelvic pain and abnormal vaginal discharge in women. However, asymptomatic courses of Ct infection are also common which often causes delays in diagnosis and treatment. Pro-longed inflammation due to pathogen persistence may result in long-term sequelae including tubal scarring and fibrosis leading to tubal infertility and ectopic pregnancy. However, the underlying mechanisms behind these pathologic processes are poorly understood and specific models that mimic the structure of fallopian tube (FT) are missing. In this study, we aimed to combine a three-dimensional (3D) fallopian-tube-on-a-chip model with green-fluorescent Ct strains to investigate Ct infections, interactions between different cell types and fibrosis mechanism.

Methods

3D fallopian-tube-on-a-chip model was fabricated in polydimethylsiloxane. The design of the device includes a channel in the central part that mimics a FT structure. The tube was created in bovine collagen type I using a needle, which was removed after the hydrogel crosslinked. The HeLa cells were seeded and

cultured inside the channel. When the cells populated the tube, the media inside the tube was replaced with media containing a green-fluorescent strain of *Chlamydia trachomatis* (Ct-GFP) to initiate infection. Ct-GFP was generated by transformation of Ct (L2 serovar) with a vector encoding the green fluorescent protein (GFP). The green signal was observed by fluorescence microscopy. Quantitative PCR and immunostaining detecting inflammatory, epithelial–mesenchymal transition and fibrosis markers were applied.

Results

We were able to create a 3D *in vitro* platform that maintains the tubular shape of the FT and can be infected with Ct, closely mimicking the conditions of a naturally occurring infection. We obtained productive infection in the tube without cell centrifugation or cycloheximide intervention, which is often used to enhance Ct infection in 2D cell culture models. The utilization of Ct-GFP enabled us to follow infection progression for an extended period of time to observe changes associated with chronic infection and fibrosis. We tracked the spread of infection, starting from the initial part of the tube, where media containing Ct was injected and Ct inclusions appeared first. Gradually, the fluorescent signal became visible subsequently in the middle and distal part of the tube. Culturing infected cells inside the device resulted in increased expression of inflammatory and fibrosis markers.

Conclusions

Our proposed 3D fallopian-tube-on-a-chip model enables to study Ct infection, progression and long-term effects, such as Ct-induced fibrosis. It provides a promising alternative for 2D cell culture and animal studies, with the benefit of tracking how infection progresses over time in a 3D environment. Additionally, replacing HeLa component with human FT cells promises better insight into Ct pathogenesis and tubal fibrosis. Future work includes expanding the library of fluorescently labelled Ct genotypes to monitor the infection dynamics of clinically relevant strains. This opens up opportunities for new applications e.g. investigating the genetic background of fibrosis, such as TRL4 point mutations, and screening of candidate anti-Ct therapeutics.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_519 Sprayable Multi-Metal Ion-Releasing Hydrogel: Advancing Wound Healing Inspired by Body's Own Natural Repair Mechanisms. Doyel Ghosal[1], Sachin Kumar[2]

[1]*Centre for Biomedical Engineering, IIT Delhi, Hauz Khas, New Delhi, Delhi 110016, New Delhi, India*; [2]*Centre for Biomedical Engineering, Indian Institute of Technology, New Delhi 110016, India, Department of Biomedical Engineering, All India Institute of Medical Sciences, New Delhi 110029, India, New Delhi, India*

Introduction/Objectives

Wound healing is a complex process involving hemostasis, inflammation, proliferation, and remodeling, which rely on factors such as cell migration, antibacterial defense, oxidative stress regulation, macrophage polarization, collagen deposition, and vascular regeneration. Despite advancements, challenges like prolonged healing, infections, and inefficient tissue repair persist. Metal ions, including calcium (Ca^{2+}), copper (Cu^{2+}), and zinc (Zn^{2+}), play essential roles in healing by aiding hemostasis, cell migration, angiogenesis, antibacterial activity, tissue repair, and oxidative stress reduction. However, existing wound care products often fail to replicate the sequential release of these ions, limiting their therapeutic effectiveness. To address this, a biocompatible sprayable hydrogel system was developed, composed of alginate (Alg), fibrin (Fib), and polylactic acid (PLA) porous microbeads loaded with multi-metal oxide nanoparticles. Termed Alg-Fib-PLA-nCMB, this hydrogel provides controlled and sustained release of Ca^{2+}, Cu^{2+}, and Zn^{2+} ions, supporting the natural healing process. This study aimed to design and evaluate this

sprayable hydrogel system to mimic natural healing mechanisms and enhance wound repair by promoting cell migration, antibacterial activity, oxidative stress reduction, macrophage polarization, collagen deposition, and vascular regeneration.

Methods

The hydrogel, termed Alg-Fib-PLA-nCMB, was formulated using alginate (Alg) and fibrin (Fib), reinforced with polylactic acid (PLA) porous microbeads loaded with multi-metal oxide nanoparticles. Its structural properties, including porous morphology and storage modulus, were characterized to confirm similarity to native extracellular matrix (ECM). The hydrogel's degradation profile and ion release over 25 days were quantified, and its bioactivity was validated through in vitro assays. In vivo experiments in a rat wound model compared its therapeutic efficacy to a commercial product (Midstress spray). Histological analysis assessed collagen deposition, epidermis formation, and vascular regeneration. The hydrogel's hemostatic potential was tested in a rat tail incision model.

Results

The Alg-Fib-PLA-nCMB hydrogel exhibited controlled and sustained release of calcium, copper, and zinc, supporting key healing activities. In vitro studies confirmed its biocompatibility and bioactivity, while in vivo results demonstrated accelerated wound closure. Complete healing was observed within 6 days for hydrogel-treated wounds, compared to 12 days for Midstress spray-treated wounds. Histological analyses revealed enhanced collagen deposition, macrophage polarization towards the M2 phenotype, and improved vascular regeneration in hydrogel-treated wounds. Additionally, the hydrogel demonstrated instant hemostatic potential by effectively preventing blood loss upon rat tail incision.

Conclusions

The Alg-Fib-PLA-nCMB hydrogel represents a significant advancement in wound care by integrating a multi-metal ion delivery strategy into a biocompatible and biodegradable system. By mimicking the body's endogenous healing mechanisms, the hydrogel effectively activated host-specific progenitor cells, facilitating all phases of wound healing. Its sprayable form and enhanced therapeutic performance provide an innovative, practical solution for improving wound care in clinical settings.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_660 Macromolecular crowding allows for the accelerated development of full thickness skin substitutes. Laura Trujillo Cubillo[1], Dimitrios Zevgolis[1]

[1]*Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Charles Institute of Dermatology, Conway Institute of Biomolecular & Biomedical Research, School of Mechanical & Materials Engineering, University College Dublin (UCD), Dublin, Ireland*

Introduction/Objectives

The development of tissue engineered full thickness skin substitutes (FTSSs) requires long culture times and high manufacturing costs, ultimately hampering benchtop-to-clinic translation. By incorporating macromolecular crowding (MMC) into the developmental cycle of FTSSs, we recapitulate *in vitro* the *in vivo* crowded microenvironment, where biological processes occur rapidly. As a result, under MMC conditions, FTSSs can be manufactured within days, as opposed to weeks that traditional non-MMC approaches require. Here we explored the use of MMC combined with a collagen scaffold to produce a biologically relevant FTSSs.

Methods

Commercially available collagen scaffolds were seeded with human dermal fibroblasts (hDFs) and human epidermal keratinocytes (hEK) and cultured without and with MMC. Dermal substitutes were

subjected to histological analysis for collagen I, collagen III, fibronectin, elastin and epidermal markers (e.g. keratin, loricrin, filaggrin). Molecular analysis (e.g. growth factor retention, inflammation, remodelling) was conducted with appropriate ELISA kits.

Results

Collagen scaffolds had appropriate porosity to allow for cell infiltration. None of the scaffolds assessed affected basic cell function. MMC enhanced and accelerated ECM deposition, without jeopardising basic cell function. MMC also increased growth factor retention, decreased inflammation and promoted remodelling in the developed FTSSs. Histological analysis is under way.

Conclusions

This work advocates the use of MMC in the development of FTSSs using commercially available collagen devices as templates for cell (hDFs and hEK) growth.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_1002 Decellularized Placental Tissue Derived Bilayer Wound Dressing. Tuna Ozurun[1], Halime Kenar[2]

[1]*Acibadem University, Biomaterials Center, Department of Biomedical Engineering, Istanbul, Türkiye*; [2]*Acibadem University, Biomaterials Center, Department of Biomedical Engineering, Natural and Applied Sciences, Istanbul, Türkiye*

Introduction/Objectives

This study aims to develop a wound dressing using placental tissues to treat chronic, deep tunneling wounds in diabetic patients. The process involves decellularizing the placental disc and its enzymatic digestion to obtain a pre-hydrogel, to be modified with methacrylate for rapid gelation. Additionally, the decellularized placental membrane will serve as a cover to protect and seal the wound. The goal is to create a wound dressing that can conform to the contours of irregularly shaped wounds and that possesses the angiogenic and anti-inflammatory properties of placental tissues to promote faster and effective healing in chronic wounds.

Methods

Both the placental disc and membrane were subjected to freeze-thaw cycles. The membranes were stretched on 3D printed stretchers and the placental disc was minced into small pieces to assist decellularization. Two different detergents (SDS and Triton-X100) were utilized to remove cells from the tissues. DNAse enzyme was used to remove the residual DNA. Staining with DAPI and measurement of double stranded DNA (dsDNA) in extracellular matrix (ECM) were performed to show absence of nuclear material and evaluate the success of decellularization. Sulfated glycosaminoglycan (sGAG) content of the decellularized tissues was determined after digestion within pepsin. Scanning electron microscopy (SEM) was used to morphologically analyze the decellularized membrane. After the decellularization the placental disc was digested with pepsin/HCl solution to obtain a pre-hydrogel. Cytocompatibility of the hydrogel and the membrane were tested with human dermal fibroblasts and human umbilical vein endothelial cells (HUVEC). Both the pre-hydrogel and the decellularized membrane were modified with Methacrylic anhydride (MA).

Results

With the decellularization process, dsDNA was reduced to 61.5 ng/mg dry weight in the membrane ECM and 70 ng/mg dry weight in the disc ECM. Cell nuclei were not observed in confocal micrographs. Amount of sGAG was determined as 3.5 μg/mg dry weight in the decellularized membrane and 3.1 μg/mg dry weight in the pepsin digested disc ECM. Thermal gelation of the neutralized pre-hydrogel was observed at 37^{o}C.

Both HUVEC and fibroblasts were observed to adhere and proliferate on the decellularized membrane and the hydrogel, separately. Methacrylation of the pre-hydrogel did not cause cytotoxicity in fibroblasts after photo-crosslinking with LAP at 405 nm.

Conclusions

The membrane and the disc were successfully decellularized and modified with methacrylate. Human dermal fibroblasts and HUVEC were able to adhere to and proliferate on both decellularized membrane and the hydrogel. Methacrylation of the pre-hydrogel did not cause cytotoxicity in fibroblasts after photo-crosslinking. Diabetic wound healing tests need to be performed at least under in vitro conditions, to comment on effectiveness of the wound dressing in treatment of such chronic wounds.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_1200 Bi-functional, non-adhesive patches for advanced treatment of skin diseases and chronic wounds using bacteriophages and antioxidant natural compounds. Alexandra Soto-Prado[1], Federica Frenna[1], Carole Armal[2], José Carlos Rodríguez-Hernández[1], Agnès Mihajlovski[2], Guillermo Vilariño-Feltrer[1], Emmanuel Pauthé[2], Ana Vallés-Lluch[1 3], Rubén Martín Cabezuelo[4]

[1]Centre for Biomaterials and Tissue Engineering, Universitat Politècnica de València, Valencia, Spain; *[2]Equipe de recherche sur les relations matrice extracellulaire-cellules, CY Cergy Paris Université, Cergy-Pontoise, France*; *[3]Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valencia, Spain*; *[4]Centre for Biomaterials and Tissue Engineering, Valencia, Spain*

Introduction/Objectives

The main goal of this work is to develop an elastic patch that can be applied topically on focalized skin sites to treat the clinical signs attributed to chronic wounds or diseases such as atopic dermatitis or psoriasis. The patch is intended to be used as a reservoir for the controlled release of bacteriophages, in order to address Staphylococcus aureus infections or biofilms, and of bioactive molecules, to minimize the inflammatory response.

Methods

Salt-leaching was selected for producing scaffolds made of the biocompatible elastomer poly(glycerol sebacate) (PGS). Salt grains of different size were used to create a stratified porous structure to prevent adhesion of skin cells. Different hydrogel formulations were studied and gelled in situ inside the cavities of the scaffold to regulate skin hydration, but also as a vehicle to mediate in the release of the agents that provide the bi-functional trait to the patch. Namely, a cocktail of GRCS and ISP bacteriophages were selected because of their specificity to fight methicillin-resistant S. aureus (MRSA), and curcumin was used for its established antioxidant effect in human tissues. Due to its hydrophobic nature, curcumin was encapsulated in nanostructured lipid carriers (NLCs) that can also enhance its therapeutic action.

Results

The stratified scaffolds made of PGS proved to be effective as an elastic, protective platform to house the rest of the components, while not interfering in their effects, since PGS is chemically stable once cured and barely swells in contact with water. From all alternatives tested, alginate (Alg), poly(vinyl alcohol) (PVA) and quaternized chitosan (HTCC) hydrogels provided the best performance to form stable gels within the pores of the patch without reducing significantly the potential infectivity of dwelling bacteriophages. PVA cryogels stood out for its rheological consistency, while alginate excelled in terms of manipulability and easy-processing. Also, using HTCC in hydrogels allows to exploit its well-documented antimicrobial properties. The hydrogel matrices avoided a burst release of the phages and the curcumin (only 10-30%

available at 24 h), thus ensuring a persistent antibiotic action, especially for ISP bacteriophages, even though MRSA are known to become resistant to phage therapy over time. On the other side, the NLCs enabled the curcumin to be biologically-active in the cell cytoplasm while the long-standing presence in the medium boosted its antioxidant activity.

Conclusions

A novel strategy to treat skin local skin diseases and chronic wounds has been developed as a multi-scale platform able to tackle with challenges such as flexibility for application in different skin sites, mediate in the water balance of the surface, and extend the therapeutic time span of bacteriophages and natural bioactive compounds. While preclinical validation of its safety in relevant models is pending, preliminary tests on the efficacy of the bi-functional patch encourage scaling it up to higher tiers of technological readiness.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_1277 Hydrophilic activated carbon-based antimicrobial polyurethane foam for infected wound healing. Jay Hind[1], Atharva Poundarik[1], Anwesha Mukherjee[1], Varun Rathi[1], Bodhisatwa Das[1], Tarush Gupta[2], Pankaj Yadav[3]

[1]Indian Institute of Technology, Ropar, Ropar, India; *[2]PGIMER, Chandigarh, Chandigarh, India*; *[3]Sheela Foam Ltd, India, Noida, India*

Introduction/Objectives

Chronic ulcers have emerged as a substantial burden on patients and healthcare system, affecting millions globally each year. Treatment of infected wounds escalates wound management costs, for which low-cost antimicrobial and biocompatible dressings are needed. The use of excessive amounts of metallic antimicrobial agents (silver, copper, zinc) in a dressing for superior antimicrobial properties has been linked to cytotoxicity, susceptible to antimicrobial resistance, and a rise in dressing costs. Instead of metallic antimicrobial agents, activated carbon (AC) can be a viable option for controlling the infection without triggering antimicrobial resistance, as well as for its superior properties like adsorption capacities for wound debris, bacterial species, bacterial toxins, and their volatile amines (odor). The combination of antimicrobial agents and AC can be an option for developing a low-cost, effective wound management dressing. In this study, the emphasis is on the development of low-cost dressings having effective antimicrobial and cell viability characteristics.

Methods

A low amount of silver agent (20 mg/100 cm^2) and AC were incorporated during in-situ polyurethane foam synthesis. Foam dressings with various compositions of AC-Ag^+ (1, 2 wt.% of AC) were synthesized by using a safer hexamethylene diisocyanate-based prepolymer unlike frequently utilized diisocyanates like TDI and MDI. In vitro, characteristics of the PU Ag+-AC foams were evaluated as per BS EN 13726 standards such as free swell absorptive capacity (FSAC), and moisture vapor transmission rate (MVTR) of thermoplastic PU film.

Results

PU (2 wt% AC-Ag^+) foam showed higher antimicrobial activity compared to other groups against Staphylococcus aureus and Pseudomonas aeruginosa while maintaining cell viability (>70%). Physiochemical and mechanical properties of PU AC-Ag^+ foams were found to be enhanced and favorable for wound healing.

Conclusions

Activated carbon with silver antimicrobial PU foam dressing can be an effective treatment option for wounds infected with antibiotic-resistant bacterial pathogens whilst improving on other functional attributes such as cellular biocompatibility, exudate management, and odor absorption.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_1336 Controlled release of antimicrobial peptides from nanocellulose wound dressings for treatment of wound infections. Elisa Zattarin[1], Zeljana Sotra[2], Emanuel Wiman[3], Yağmur Baş[4], Jonathan Rakar[5], Linn Berglund[4], Annika Starkenberg[5], Emma Björk[6], Hazem Khalaf[3], Kristiina Oksman[4], Torbjörn Bengtsson[3], Johan Junker[5], Daniel Aili

[1]Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden; *[2]Center for Disaster Medicine and Traumatology, Department of Biomedical and Clinical Sciences, Linköping University, Linkoping, Sweden*; *[3]Department of Microbiology, Immunology and Reproductive Science, School of Medical Sciences, Örebro University, Örebro, Sweden*; *[4]Division of Materials Science, Department of Engineering Sciences and Mathematics, Luleå University of Technology, Luleå, Sweden*; *[5]Center for Disaster Medicine and Traumatology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden*; *[6]Division of Nanostructured Materials, Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping, Sweden*

Introduction/Objectives

Wound infections are a major clinical challenge, often leading to severe complications and impeding the normal healing process, thereby resulting in chronic, non-healing wounds. These wounds impose a profound physical and psychological burden on patients while significantly increasing healthcare costs. Effective and timely treatment of wound infections is imperative; however, the escalating prevalence of antibiotic-resistant pathogens complicates infection management. This study aims to evaluate the antimicrobial efficacy of the potent two-peptide bacteriocin PLNC8 αβ for treating wound infections and to develop a localized delivery system using advanced functionalized nanocellulose (NC) wound dressings.

Methods

The in vivo efficacy of PLNC8 αβ was evaluated using an infected porcine wound model. To develop a system for controlled release of PLNC8 αβ, two types of nanocellulose-based wound dressings were investigated: bacterial cellulose (BC) and TEMPO-oxidized nanocellulose derived from hardwood (TC). To endow the dressings with antimicrobial properties, they were functionalized with PLNC8 αβ. Functionalization with PLNC8 αβ was achieved by either direct physisorption onto the nanocellulose fibrils or by encapsulation within mesoporous silica nanoparticles (MSNs), which were integrated into the BC dressings.

Results

In a porcine wound infection model, PLNC8 αβ effectively eradicated bacterial infections while promoting re-epithelialization. The PLNC8 αβ functionalized dressings demonstrated low cytotoxicity toward human primary fibroblasts and keratinocytes. Both BC and TC dressings demonstrated robust contact-based antibacterial activity but exhibited limited efficacy against bacteria in suspension. MSN-functionalized BC dressings significantly improved peptide-loading capacity and provided sustained release of PLNC8 αβ, leading to superior antimicrobial performance.

Conclusions

This study underscores the potential of PLNC8 αβ for treatment of wound infections and demonstrates PLNC8 αβ functionalized nanocellulose wound dressings as an innovative therapeutic approach for

managing infected wounds. By combining potent antimicrobial activity with advanced delivery mechanisms, this platform offers a promising alternative to conventional antibiotic treatments, addressing the growing challenge of antibiotic resistance.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_1461 Seeding Microvascular Fragments and Fibroblasts onto PLLA Scaffolds for Chronic Wound Treatment. Francesco La Monica[1], Simona Campora[1], Alessandra Lo Cicero[1], Camilla Carbone[1], Veronica Schiera[1], Gabriele Lo Buglio[1], Gundula Schulze-Tanzil[2], Francesco Carfì Pavia[1], Giulio Ghersi[1]

[1]*University of Palermo, Palermo, Italy*; [2]*Paracelsus Medical University, Nuremberg, Germany*

Introduction/Objectives

Chronic wounds, commonly referred to as ulcers, pose a significant healthcare challenge, as current medical treatments often fail to achieve complete healing. This project aims to address this issue by developing a regenerative medicine-based strategy that utilizes PLLA (Poly-L-lactic Acid) scaffolds seeded with primary dermal fibroblasts (RDFs) and microvascular fragments (ad-MVFs) to accelerate wound healing in a murine model. To explore potential human applications, primary human dermal fibroblasts (hDFs) were also included in the experiments.

Methods

PLLA scaffolds were synthesized using the Thermally Induced Phase Separation (TIPS) technique. Primary dermal fibroblasts were isolated from both rat and human dermis, characterized via immunostaining, and seeded onto PLLA scaffolds. Additionally, a protocol to isolate microvascular fragments (ad-MVFs) from adipose tissue was optimized. Ad-MVFs were characterized through Scanning Electron Microscopy (SEM) and cultured in 2D systems (Petri dishes) and 3D environments (type I collagen matrices and PLLA scaffolds). Proliferation, morphology, and scaffold integration of both cell types were assessed using a combination of viability assays, Life/Death assays, Scanning Electron Microscopy (SEM), histology staining, and immunohistochemistry.

Results

Viability and Life/Death assays confirmed successful adhesion and proliferation of primary fibroblasts within the PLLA scaffold structure. Histological and immunohistochemical analyses of scaffold cross-sections further demonstrated that cells not only colonized the surface of the scaffold but also infiltrated its inner regions. Ad-MVFs exhibited robust growth in both 2D and 3D systems, forming vessel-like structures that interconnected to create vascular networks.

Conclusions

Primary dermal fibroblasts and microvascular fragments successfully grew within PLLA scaffolds, posing the basis for a combined approach to enhance chronic wound healing. In this context, fibroblasts would contribute by releasing growth factors and extracellular matrix (ECM) components to support tissue regeneration, whereas ad-MVFs would ensure oxygen and nutrient delivery through vascular connections to the host system. Future in vivo studies in murine models will evaluate the construct’s potential to improve wound healing outcomes, providing a promising alternative to existing treatments.

Topic: Tissue-Specific Focus

Subtopic: Wound healing

Type: Poster presentation

TERMIS25_1534 CD200-CD200 receptor interactions in healthy skin wound healing and diabetic foot ulcer. Fabiana Ziliani[1], Dominic Ruetsche[1], Katarzyna Michalak-Micka[1], Thomas

Biedermann[1], Ueli Moehrlen[2], Agnes Klar[1]

[1]Tissue Biology Research Unit, Department of Surgery, University Children's Hospital Zurich, Zurich, Switzerland, Children's Research Center (CRC), University Children's Hospital Zurich, Zurich, Switzerland, University of Zurich, Zurich, Switzerland, Schlieren, Switzerland; *[2]The Zurich Center for Fetal Diagnosis and Therapy, University of Zurich, Zurich, Switzerland, Spina Bifida Center, University Children's Hospital Zurich, Zurich, Switzerland., Zurich, Switzerland*

Introduction/Objectives

Wound healing is a complex physiological process that is vital to restore skin tissue function. However, conditions such as diabetes can impair this process leading to chronic, non-healing wounds like diabetic foot ulcer (DFU). CD200 is a membrane glycoprotein that is ubiquitously expressed on endothelial cells in human skin, whereas its cognate receptor, CD200R, is exclusively expressed on myeloid cells such as granulocytes, monocytes/macrophages, T cells in human peripheral blood. Our aim was to investigate CD200-CD200R pathway in healthy and diabetic skin wound healing.

Methods

We characterized CD200–CD200R interactions in human adult/juvenile, fetal skin, DFU, and in *in vivo* prevascularized skin substitutes (vascDESS) prepared by co-culturing human dermal microvascular endothelial cells (HDMEC), containing both blood (BEC) and lymphatic (LEC) endothelial cells (EC).

We performed flow cytometric, immunofluorescent, and qRT-PCR analyses to compare the expression of CD200 and pro-/anti-inflammatory chemokines in BEC and LEC of adult/juvenile, fetal skin, and DFU. Moreover, we used our *in vivo* animal model to investigate the CD200-CD200R interactions in vascDESS. One- and three-weeks post-transplantation onto immune-compromised rats, vascDESS skin substitutes were immunostained for CD200-CD200R.

Results

We detected the highest expression of CD200 on lymphatic capillaries of healthy adult/juvenile, fetal skin, as well as DFU, whereas it was only weakly expressed on blood capillaries. Further, the highest CD200 levels were detected on LEC showing enhanced Podoplanin expression, while reduced expression was observed on Podoplanin-low LEC. In addition, qRT-PCR analysis revealed upregulated expression of some chemokines, including CC-chemokine ligand 21 (CCL21) in $CD200^+$ LEC, as compared to $CD200^-$ LEC. The expression of CD200R was mainly detected on myeloid cells such as granulocytes, monocytes/macrophages, T cells in human peripheral blood, and human and rat skin. Functional immunoassays demonstrated specific binding of skin-derived $CD200^+$ HDMEC to myeloid $CD200R^+$ cells *in vitro*. Importantly, we confirmed enhanced CD200–CD200R interaction in vascDESS *in vivo*.

Conclusions

Our findings reveal spatially varying distribution pattern of CD200 in the blood and lymphatic endothelia both *in vivo* and *in vitro*. Functional assessments of endothelial CD200 suggested that the physical binding between CD200 and CD200R was involved in T-cell adhesion to the endothelium. We concluded that the CD200–CD200R axis plays a crucial role in regulating tissue inflammation during healthy and pathological skin wound healing.

Topic: Submit to SYMPOSIUM

Subtopic: Cell-Assembled extracellular matrix as innovative scaffold for tissue engineering: from concept to clinic

Type: Poster presentation

TERMIS25_140 Personalized cartilage reconstruction: from patient to patient. Christelle BERTSCH[1], Eya Aloui[1], Adrien Moya[2], Arnaud Scherberich[2], Philippe Lavalle[1], Léa Fath[1 3]

[1]*Inserm UMR_S 1121, Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg, 67000 Strasbourg, France, Strasbourg, France*; [2]*University of Basel - Department of Biomedicine, Basel, Switzerland*; [3]*Service d'ORL et de Chirurgie Cervico-Caciale, Hôpitaux Universitaires de Strasbourg, 1 avenue Molière 67200 Strasbourg, STRASBOURG, France*

Introduction/Objectives

Cartilage is a dense, avascular, aneural connective tissue embedded in a dense extracellular matrix (ECM) with sparsely distributed chondrocytes. Its ECM is rich in collagen type II, proteoglycans, and sulfated glycosaminoglycans (sGAG). However, it has a low self-regeneration capacity following damage. Several strategies have been used to regenerate it. No engineered replacement has fully replicated the properties and the structure of native cartilage. In general, cartilage tissue engineering (TE) requires a three-dimensional (3D) matrix to address the effective regeneration of cartilage. Natural materials, particularly proteins, have attracted great attention for TE due to their excellent biocompatibility and biodegradability, low immunogenicity, and availability on a large scale and at low cost. Albumin is the most abundant protein in plasma; it is sustainable, cost-effective, and, above all, autologous. To date, its use as a scaffold for cartilage TE has not yet been well established.

Methods

Recently, we demonstrated the formation of albumin-based materials by salt-assisted compaction (patent no. EP19306387). It is a very simple process based on the evaporation at 37°C of an albumin solution in the presence of salt. The major advantages of this process are that it does not require extreme temperatures or pH levels, which most of the time lead to irreversible denaturation of the protein, nor cross-linking agents. In addition, the production method is versatile enough to produce a material whose shape, size and various other properties can be easily adjusted to meet the requirements of TE applications. We fabricated a bilayered albumin-based scaffold for cartilage tissue engineering using a salt-assisted compaction process. The bilayered scaffold was seeded with human nasal chondrocytes (HNCs). HNCs were cultivated for 1 week with a proliferation medium to have a well-colonized scaffold before differentiation. HNCs were cultivated for 4 weeks with a chondrogenic medium.

Results

Our albumin-based scaffold comprises two layers: a smooth layer allows to maintain the original geometry of the scaffold, and a porous layer provides a 3D support for chondrocytes. To reproduce *in vitro* cartilage, we used and seeded HNCs on the scaffold. We aimed to assess whether autologous albumin scaffold seeded with HNCs allows homogeneous, mature, and viable regeneration of hyaline cartilage. The current study shows that the scaffold is biocompatible: it exhibits no cytotoxic effect and maintains high cell viability over several periods of proliferation and. Their results demonstrated that the porous layer significantly promote the formation of cartilage tissue both *in vitro* and *in vivo* (with vascularization). HNCs show homogeneous, mature, and viable hyaline-like cartilage structure with abundant cartilage-specific ECM deposition. The tissue engineering cartilage naturally reproduces *in vivo* cartilage structure and organization.

Conclusion

In conclusion, we fabricated and proposed to the literature a new albumin-based scaffold as a matrix for TE, especially for cartilage TE. This scaffold could be extended to a wide range of medical applications, such ENT, orthopedics or plastic surgery. Our study's unique innovation lies in creating fully autologous scaffolds - with patient-derived chondrocytes - to meet cartilage TE needs and advance clinical translation for patient benefit.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_370 Engineered patient-derived osteoarthritic synovial organoids induce cartilage degradation. Atharva Damle[1], Andre Tiaden[1 2], Stavros Giaglis[1 2], Petros Ismailidis[2], Florian Imhoff[2], Diego Kyburz[1 2], Andrea Barbero[1 2]

[1]*University of Basel, Basel, Switzerland*; [2]*Universitätspital Basel, Basel, Switzerland*

Introduction/Objectives

Osteoarthritis (OA) is a multi-etiological degenerative disorder characterised by cartilage degradation, synovitis, and compromised joint integrity. Current treatments for OA, however, do not contribute to articular cartilage regeneration, therefore arthroplasty is the only available treatment in advanced stages. OA joints are characterised by a chronic inflammatory environment, with the synovium contributing to cartilage breakdown through proinflammatory cytokines. Despite its clinical prevalence, the mechanisms underlying OA pathogenesis remain poorly understood, particularly concerning the dynamic interplay between immune cells, synovial fibroblasts, and cartilage in the joint microenvironment. Traditional in vitro models fail to replicate the complex interactions observed in human OA joints, limiting the efficacy of preclinical drug testing. To address these gaps, we have developed patient-derived OA synovial organoids as a novel 3D in vitro model that faithfully recapitulates the synovial environment of OA. By utilising primary cells obtained from OA patients, these organoids mimic key pathological features of the disease.

Methods

BMSCs were isolated from three healthy donors. Synovial cells, including fibroblast-like synoviocytes (FLS), were isolated from synovial tissue specimens from OA donors undergoing knee replacement. BMSCs were differentiated into micro-cartilage spheroids that were cultured for an additional two weeks under inflammatory conditions. These conditions included hypertrophic medium (OACart), as well as conditioned media (CM) from IL1β (10ng/ml) activated OA synovial organoids in two forms: (i) patient-derived (P0-Syn) and (ii) coculture of FLS with THP-1 cells (FLS+THP-1). A separate group was exposed to a medium containing 500 pg/mL of recombinant IL-6. Unstimulated spheroids served as controls. All cultures were harvested after the respective culture periods for histological, ELISA and RT-qPCR.

Results

Histological analysis of the unstimulated cartilage organoids (- INFL) revealed strong and uniform staining for GAG, COL2, weak staining for COL10, and faint/scattered staining for MMP13, overall indicating the hyaline-like feature of these tissues. Cartilage organoids exposed to inflammatory stimuli demonstrated significant cartilage degradation, with a substantial reduction of cartilage matrix proteins, more pronounced at the periphery. Notably, cartilage organoids exposed to CM of activated P0-Syn organoids showed high MMP13 expression (at protein and mRNA levels), indicating elevated cartilage catabolism, similar to the one observed in OACart. ELISA analyses on supernatant showed that IL-8 was significantly higher in OACart (4.8 ng/mL) and P0-Syn-CM (4.2 ng/mL) stimulated organoids compared to controls. Additionally, gene expression analysis highlighted an upregulation (vs unstimulated spheroids) of inflammatory and catabolic markers in P0-Syn-CM-stimulated cartilage organoids, such as IL-6 (487-fold), IL-8 (245-fold), PTGS2 (5.6-fold), and MMP13 (16-fold), which level approaching the ones quantified in OACart.

Conclusions

Patient-derived synovial OA organoids (P0-Syn) induce cartilage organoids to acquire inflammatory degrading features. Co-culture of cartilage and P0-Syn would then represent a viable model to study cross-talks among cartilage and synovium in an OA setting and to assess the therapeutic effects of novel anti-OA molecules.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_404 Mechanically enhanced tissue-engineered cartilage regeneration and exploratory clinical translational research. Zheng Ci[1], Guangdong Zhou[2]

[1]Shanghai Pulmonary Hospital, Shanghai, China; *[2]Shanghai Key Laboratory of Tissue Engineering, Shanghai, China*

Introduction/Objectives

Cartilage defects are common in clinical practice and difficult to treat. At present, autologous cartilage transplantation is still the first choice, but the source of autologous cartilage is limited and the injury is irreversible. Tissue engineering provides a new approach to this challenge. Decalcified bone matrix (DBM) is a natural scaffold material, but due to its large pore size, it is difficult to be directly used for cartilage regeneration and repair.

Methods

The engineered cartilage gel (ECG) was prepared and seeded on the DBM scaffold; DBM-gelatin composite scaffold was prepared and seeded cells (chondrocytes/BMSCs) to regenerate cartilage *in vitro*; And the subcutaneous cartilage regeneration and osteochondral defect regenerative repair were successfully achieved; Subsequently, it was successfully applied to the clinical treatment of a variety of osteochondral deformities/defects.

Results

Successfully prepared ECG with good flow and adhesion ability, and combined with DBM to subcutaneously regenerate cartilage in large animals; After precise modification of DBM pore size with gelatin, the pore structure and cell adhesion rate were effectively improved, and successfully achieved subcutaneous mechanically enhanced cartilage regeneration and osteochondral defect repair; Exploratory clinical translational research of mechanically enhanced cartilage regeneration technology has been carried out in many high-level hospitals.

Conclusions

Successfully established mechanically enhanced tissue-engineered cartilage regeneration technology and launched exploratory clinical translational research, providing ideal tissue regeneration grafts for patients with various high-strength mechanical demands for cartilage and osteochondral defects.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_418 Development and characterization of a bovine articular cartilage explant model to examine the effects of adipose tissue secretome. Zahra Sarmadian[1], João Henriques[1], Valtteri Peitso[1], Maija Risteli[1], Ali Mobasheri[1 2 3]

[1]Research Unit of Health Sciences and Technology, Faculty of Medicine, University of Oulu, Oulu, Finland; *[2]State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania*; *[3]Department of Joint Surgery, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China*

Introduction/Objectives

Osteoarthritis (OA) is a highly prevalent joint disease linked to morbidity and disability globally. Rising obesity rates and an ageing population are anticipated to increase OA prevalence. Although obesity is a major risk factor for OA, the mechanisms behind disease progression and the adipose tissue secretome impact on the extracellular matrix (ECM) of articular cartilage remain poorly understood. This study aimed to develop an explant model of bovine articular cartilage to investigate the effects of adipose tissue-derived factors on cartilage, including those that simulate obesity-related inflammation from obese adipose tissue, or those that promote cartilage repair and regeneration through the action of secreted anabolic and repair factors from healthy (non-obese) adipose tissue.

Methods

Fresh bovine knees were purchased from a local abattoir. Infrapatellar fat pad (IPFP) and cartilage explants were harvested and prepared for the study. IPFP tissue was sectioned into ∼10 mg pieces and cultured for 24 hours in medium containing Dulbecco's Modified Eagle's Medium, ITS (10 mg/ L insulin, 55 mg/L transferrin, 6.7 mg/L selenium), penicillin/streptomycin/amphotericin, 4 mM L-proline, 50 µg/mL L-ascorbic acid, 10 mM HEPES, 10 mM MEM Non-Essential Amino Acids solution. The adipose-conditioned medium (ACM) was collected for use in the bovine cartilage explant model. Cartilage explants (5 mm diameter, 1 mm thick) were equilibrated for 24 hours and treated for five days under four conditions: (1) control (same medium as used for ACM collection), (2) pro-inflammatory cytokines (control medium + 10 ng/ml IL-1β + 5 ng/ml TNF-α), (3) ACM, and (4) ACM + pro-inflammatory cytokines. Media were analyzed for glycosaminoglycan (GAG) release (DMMB assay), ECM protein levels (SDS-PAGE, followed by Coomassie blue and silver staining), and matrix metalloproteinase (MMP) activity (zymography).

Results

Pro-inflammatory conditions (cytokines and ACM + cytokines) increased GAG release from cartilage explants compared to untreated controls. However, in the presence of ACM, less GAG was released into the media. Zymography revealed the presence of pro-MMP2 and pro-MMP9 in all samples, with higher levels of active MMP2 and MMP9 in the ACM and ACM + cytokine conditions. ACM alone exhibited high levels of active MMP2 and MMP9 before exposure to cartilage explants.

Conclusions

This study demonstrates that stimulation with pro-inflammatory cytokines increases GAG, ECM protein, and MMP release from cartilage explants. The IPFP secretome likely contains some factors that simulate obesity-related inflammation as well as anabolic and protective factors that may serve as mediators of joint preservation. This explant model provides a platform for further investigating the immunometabolic effects of adipose tissue-derived pro-inflammatory and anti-inflammatory factors on articular cartilage and testing novel therapeutic interventions.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_536 Engineering mechanically functional cartilage through growth-induced collagen alignment. Jet Peters[1], Florencia Abinzano[1], Aref Saberi[2], Sebastien Callens[1], Keita Ito[1]

[1]*Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands, Eindhoven, Netherlands*; [2]*Soft Tissue Engineering & Mechanobiology, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands, Eindhoven, Netherlands*

Introduction/Objectives

Articular cartilage has a unique structural arrangement of type II collagen fibers that extends from the underlying bone into the deep cartilage zones to bend over and lay flat in the superficial zone. This distinct architecture plays a vital role in the tissue's ability to bear mechanical loads. For tissue engineering purposes it is key to accurately replicate the collagen organization to ensure proper functionality and prevent breakdown in the engineered cartilage tissues [1]. During growth of native cartilage, a transition in fiber organization occurs, changing from an isotropic arrangement after birth to the anisotropic architecture in adult cartilage. However, the mechanism governing these changes is not well understood. In a recent study, we demonstrated the potential of localized and directional growth of the tissue as a mechanism to drive collagen orientation, mimicking native postnatal growth processes. We found type II collagen fibril alignment in the direction of the growth, as in the deep zone of cartilage, when growth was localized deep in

the tissue. Building on these results, the present study aims to investigate how different types of local growth stimulation, and therefore tissue organization, influence the mechanical properties of the engineered tissues.

Methods

Custom-made PDMS micro-transwell inserts were developed, with cylindrical wells (Ø 1 mm, 2.5 mm in height). A polyethylene terephthalate membrane (0.4 μm pore size) was mounted on the bottom to create a two-compartment culture system. A suspension of cartilage organoids [2] was added to each insert. Culture medium was added to both compartments, and organoids were allowed to fuse for 7 days. Afterwards, medium was supplemented with human transforming growth factor β1 and dexamethasone in either the well or transwell compartment, for directional stimulation of growth. After 28 days, directed tissue growth was assessed with biochemical assays and staining for collagen and glycosaminoglycans Collagen fiber organization was evaluated with polarized light microscopy. Bulk mechanical properties of the samples were assessed with micromechanical compression techniques.

Results

Using microfabrication, the micro-transwell system was developed successfully, allowing culture of organoid-based microtissues. Preliminary results show fusion of the organoids after 7 days, where the spaces between the organoids are filled with new tissue. Radial confinement of the microtissue in the microwells prevents mixing of the media from well and transwell compartment, enabling local stimulation of growth, similar to our recent study.

Conclusions

We developed a novel *in vitro* culture system that enables the successful culture and fusion of organoids to create cartilage-like tissues, locally stimulated with chondrogenic factors to promote targeted growth. Ongoing work focuses on characterizing the tissues' structural organization and mechanical properties. Our system aids in the understanding of cartilage growth by effectively mimicking native growth processes to optimize their potential for functional cartilage engineering applications.

This research was financially supported by the Gravitation Program “Materials Driven Regeneration”, funded by the Netherlands Organization for Scientific Research (024.003.013).

[1] Nagel T, Kelly DJ *Tissue Eng Part A.* 2013.

[2] Crispim JF & Ito K, *Acta Biomater.* 2021.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_538 A bioprinted hyperglycemia model with adult rat chondrocytes harvested from the femoral epiphysis. Gundula Schulze-Tanzil[1], Clemens Gögele[1], Nils Fleischmann[2], Tanja Liesenberg[1], Teresa Schotte[3], Christian Werner[1], Gina Pizzadili[4 4], Maria Kokozidou[1]

[1]Paracelsus Medical University, Institute of Anatomy and Cell Biology, Nürnberg, Germany; *[2]Friedrich Alexander Universität-Erlangen, Institute für Anatomie, Lehrstuhl für Mikroskopische Anatomie und Molekulare Bildgebung, Erlangen, Germany*; *[3]Universitätsmedizin Göttingen, Klinik für Allgemein-, Viszeral- und Kinderchirurgie, Göttingen, Germany*; *[4]Paracelsus Medical University, Nürnberg, Germany*

Introduction/Objectives

Harvesting sufficient amounts of articular chondrocytes from adult rodent joints remains challenging due to the very thin joint cartilage layer. Hence, joints of many immature rats were often pooled for cell isolation e.g. for tissue engineering. Despite of joint cartilage maturation, adult rats show physiologically a delayed epiphyseal (growth) plate closure. Osteoarthritis, the main joint disease, and diabetes mellitus which is associated with hyperglycemia, are interrelated. A novel epiphysis dissection technique was established to

isolate a large amount of epiphyseal rat chondrocytes which were subsequently used to gain a bioprinted 3D hyperglycemia model.

Methods

The proximal femoral epiphysis of adult male Sprague Dawley rats used for a knee OA model were explanted *post mortem*: After medial exarticulation of the femoral head preserving the integrity of the teres ligament, the entire femoral head cartilage layer remained attached to the ligament within the acetabulum, whereas the bony metaphysis was released. The cartilaginous epiphysis, epiphyseal explant cultures and isolated epiphyseal chondrocytes were thoroughly characterized and cells exposed to TNFalpha to mimick inflammatory conditions. In addition, chondrocytes were bioprinted into 3D grids using a methylcellulose/alginate hydrogel and exposed to hyper- and normoglycemic condition. Cell vitality and gene expression of cartilage markers were analyzed after 7 and 14 days in bioprinted constructs in comparison to monolayer culture.

Results

In 87.88% of the rats (mean weight 558.84 g, age 70-153 days, n=33) the epiphysis dissection technique was successful. Epiphyses were released by breakage within the hyaline cartilaginous epiphyseal plate (Fig. 1). They consisted of hyaline cartilage with typical joint cartilage zonality. Hypertrophic calcified cartilage representing the early ossification center was detectable, but blood vessels remained barely detectable. Cultured epiphysis explants contained mostly viable chondrocytes, even after 1-3 weeks. Epiphyseal chondrocyte monolayers synthesized cartilage-specific collagen type 2, cartilage proteoglycans, the chondrogenic transcription factor SOX9, but no calcium deposition was detected. They showed significant suppression of collagen type 1 and 2 expression in response to TNFalpha. After bioprinting, cells survived in bioprinted constructs for the whole observation time of 14 days. The gene expression of aggrecan, collagen type 2, and type 1 at day 14 d was higher in bioprinted 3D constructs compared to the monolayer, irrespectively whether cultured in hyperglycemic or normoglycemic culture medium. Only type 1 collagen was suppressed by hyperglycemic conditions.

Conclusions

The technique allows to gain additional non-degenerated cartilage from adult rats simultaneously used as knee OA models, thereby substantially reducing overall animal numbers. Epiphyseal chondrocytes used as in vitro OA model reflected the typical catabolic response to TNFalpha, and could serve as 3D bioprinted diabetes mellitus model.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_1308 In vitro characterization and evaluation of the chondrogenic property of mesenchymal stem cells secretome. Lucía Bravo[1], Mauro Llop-Miguel[2], Irene Monleón-Guinot[1], María Sancho-Tello[1], Lara Milián Medina[1], Manuel Mata Roig[3]

[1]*Universitat de València, Faculty of Medicine, Pathology Department, Spain, INCLIVA, Valencia, Spain*; [2]*Universitat de València, Faculty of Medicine, Pathology Department, Spain, Valencia, Spain*; [3]*Universitat de València, Faculty of Medicine, Pathology Department, Spain, INCLIVA, CIBER-bbn, Valencia, Spain*

Introduction/Objectives

We have optimized an intensive 3D culture system for the collection of the secretome of dental pulp stem cells (hDPSC). Our aim is to characterize this secretome and study its capacity for chondrogen induction in vitro. To this end, we have analyzed the profile of mediators secreted into the culture medium using proteomics and miRNA expression, and we have stimulated primary human chondrocytes with this secretome to study the induction of chondral differentiation markers.

Methods

To obtain the secretome, we encapsulated hDPSCs in 3% alginate/4% agarose beads and cultured them for 4 weeks in chondrogenic differentiation medium in a bioreactor with a continuous culture medium replacement system. We collected the culture medium weekly and concentrated it with 30 kDa filters. We analyzed the protein expression profile by mass spectrometry and the expression of miRNAs. We stimulated primary chondrocytes cultured in 2D and in microtissues and analyzed the morphological changes generated by fluorescent actin staining. We evaluated the expression of the chondrogenic differentiation markers type II collagen and aggrecan by immunofluorescence and real time RT-PCR.

Results

Proteomic studies revealed the induction of more than 1400 expression proteins in the collected secretome. Among them we highlight TGFbeta, Serpina and Il1beta. We also detected the expression of miRNA 23a and 337-5p. Stimulation of chondrocytes with the secretome induced uncontrolled cell proliferation and notable changes in morphology.

Conclusions

The designed culture platform is capable of generating large amounts of conditioned medium with a significant content of mediators involved in chondral differentiation. This secretome induces the differentiation of primary chondrocytes and articular cells in vitro.

Topic: Tissue-Specific Focus

Subtopic: Cartilage

Type: Poster presentation

TERMIS25_1457 Histological characterization of platelet-rich plasma microgel-induced articular cartilage regeneration in a porcine experimental model of articular cartilage injury. María Sancho-Tello[1 2], Lara Milián Medina[1 3], Clara Francés[1], Joaquín Ródenas-Rochina[4], Julia Pla-Salom[4], Gloria Gallego-Ferrer[4 5], José Luis Gómez Ribelles[4 5], Manuel Mata Roig[1 3 5]

[1]Department of Pathology, University of Valencia, Valencia, Spain; *[2]INCLIVA Biomedical Research Institute, Valencia, Spain*; *[3]INCLIVA, Biomedical Research Institute, Valencia, Spain*; *[4]Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Valencia, Spain*; *[5]CIBER-BBN, Carlos III Health Institute, Valencia, Spain*

Introduction/Objectives

Articular cartilage has a poor regenerative capacity and therefore chondral lesions frequently give rise to a fibrous cartilage scar that progresses to osteoarthritis. To improve cartilage regeneration in joint lesions, mesenchymal stem cells (MSCs) have been used to induce them towards a chondral differentiation, along with scaffolds made of different materials that generate an environment that provides the necessary biomechanical forces and, finally, abundant biomolecules to induce cells to acquire a hyaline chondral phenotype. In a porcine experimental model, we previously stimulated the arrival of MSCs from the subchondral bone marrow by microdrilling the subchondral bone and filling the chondral lesion with a biomimetic microgel containing microspheres made from a mixture of platelet-rich plasma (PRP) and a biodegradable polymer, and observed biomechanical and morphological differences with respect to controls. Therefore, the objective of the present work was to further study the role of PRP administered as gelatin, as a chondral inducer, through a deep morphological study of the newly formed articular cartilage.

Methods

We used a pig model of articular cartilage injury, following previous studies. Eight mini pigs were used, in which a lesion was performed on the femoral condyles using a 7-mm diameter punch, which completely removed the articular cartilage. Three microdrillings were then performed to perforate the subchondral bone plate and thus allowing the arrival of blood with bone marrow MSCs to the injured area. Animals were

divided into 2 groups: (i) PRP group, where the cavity was filled with gelatin containing microspheres of PRP and of a biodegradable polymer, and covered with a membrane, and (ii) the control group, where the cavity was left empty and was also covered with a membrane. Animals were sacrificed 9 months after surgery, and the newly formed cartilage was evaluated by histological study, using standard techniques (hematoxylin-eosin, Masson's trichrome, and toluidine blue) as well as immunohistochemical techniques (using normal hyaline cartilage marker as type II collagen, and markers of chondral remodeling such as type X collagen, vimentin and vascular endothelial growth factor, VEGF), quantifying various morphological parameters of the cells and the components of the extracellular matrix of the newly formed tissue, followed by a statistical study.

Results

We observed a great variability between the different samples within the same group. However, PRP group frequently presented a neotissue that filled a high percentage of the injured cavity in the articular cartilage, containing abundant areas of cartilage with hyaline-like characteristics, with high glycosaminoglycans (GAGs) content (as shown with toluidine blue staining), which coincides with the areas that are positive for type II collagen immunostaining, while control group presented a lower occupation of neotissue in the injured cavity, which frequently contained a tissue with characteristics of fibrocartilage and low GAGs content, that coincides with type I collagen immunostaining.

Conclusions

PRP improves the formation of a neotissue with morphological characteristics of hyaline cartilage in an articular cartilage lesion in a porcine animal model, while in its absence the newly formed tissue occupies a smaller area and is closer to fibrocartilage-type morphology.

Topic: Translation

Subtopic: ATMPs

Type: Poster presentation

TERMIS25_298 Establishing GMP-compliant manufacturing and quality control standards for injectable nasal chondrospheres (NCS) in minimally invasive nucleus pulposus repair. Olga Krupkova[1 2], Raphael Schmid[1 2], Janhavi Apte[2], Günther Schäfer[2], Salim Darwiche[3], Karina Klein[3], Stefan Schären[1], Ivan Martin[2], Andrea Barbero[2], Karoliina Pelttari[2], Anke Wixmerten[2], Arne Mehrkens[1]

[1]Spine Surgery, University Hospital Basel, Basel, Switzerland; *[2]Department of Biomedicine, University of Basel & University Hospital Basel, Basel, Switzerland*; *[3]Musculoskeletal Research Unit (MSRU), University of Zurich, Zurich, Switzerland*

Introduction/Objectives

Current treatments for painful intervertebral disc (IVD) degeneration provide only limited relief, highlighting the need for new therapies. We have shown that preconditioned nasal chondrocyte-derived spheroids (NCS) survive and integrate into degenerated IVD in ex vivo and in vivo models, showing promise as a cell therapy. To ensure NCS consistency and safety for human use, good manufacturing practice (GMP)-compliant processes and defined quality control (QC) measures must be established to meet clinical standards.

Methods

Human and sheep nasal chondrocytes (NC) were isolated and NCS were fabricated using GMP-compliant materials. Intermediate products (IP) were characterized for inter- and intra-donor variably in size, shape, elastic modulus (MicroTester G2), viability (CellTiter-Glo, Trypan Blue), and key bioactive factors used during NCS fabrication (TGF-β3, IL-1Ra). The final product (FP) included 100 NCS from a single donor in saline. Stability of FP was assessed in terms of size, shape, viability (individual NCS), presence of bioactive factors (storage medium), and injectability. $N \geq 5$ donors and replicates were used per experiment.

Results

Research-use-only (RUO) materials were successfully replaced with human use (HU) suitable alternatives. TGF-β3 was confirmed as necessary for NCS fabrication, with a washing step efectively removing its residues. Following characterization, product specifications ensuring injectability and funcion of individual NCS were defined as (i) diameter <600µm, (ii) roundness >0.8, (iii) elastic modulus >5 kPa, (iv) cell viability >90%, (v) undetectable TGF-β3, (vi) IL-1Ra >1ng/NCS. Variability in these attributes was not significant. The FP remained stable for at least 24h at 4°C under mild agitation. Consistent results were obtained for human and sheep NCS. Human NC identity was previously confirmed (PMID: 27789021).

Conclusions

We have achieved good reproducibility and consistency in the manufacturing process. Product specifications were defined based on the obtained limits/ranges of critical QC attributes and will be used to establish in-process controls (IPC) and acceptance criteria (AC) for product release to patients. Validation of NCS GMP processes is ongoing in a sheep trial, and the relationship between the intended mode of action and efficacy is under investigation.

Topic: Translation

Subtopic: ATMPs

Type: Poster presentation

TERMIS25_931 Cryopreservation of implantable cell-microcarrier combinations for use in clinical regenerative medicine. Chara Simitzi[1], Barry Fuller[2], Rainer Marksteiner[3], Richard Day[1]

[1]Division of Medicine, University College London, London, United Kingdom; *[2]Division of Surgery, University College London, London, United Kingdom*; *[3]Innovacell GmbH, Mitterweg 24, A-6020, Innsbruck, Austria*

Introduction/Objectives

Advanced therapy medicinal products (ATMP) often rely on centralized manufacturing to support essential global distribution. Duration of the supply chain can be particularly challenging when using cells that require anchorage to other cells and/or matrices in the body. Therefore, bioprocessing, storage, transport, and handling conditions have the potential to negatively impact the ATMP functionality and require the development of entirely new manufacturing and distribution paradigms. To deliver anchorage dependent cells, such as skeletal muscle derived cells (SMDC), in a more natural optimal anchored state, we have developed novel implantable microcarriers for cell transplantation, which are being investigated for treatment of incontinence (www.amelie-project.eu). The product has a short shelf-life, which makes shipping in a fresh format logistically challenging.

This study investigated the feasibility of cryopreserving the implantable cell-microcarrier combination to enable cold chain supply of the product. The specific objectives were: i) to determine the cell viability and adhesion post-thawing for different cryopreservation parameters, ii) to evaluate the myogenic differentiation of the cells post-thawing for different cryopreservation parameters and iii) to establish a cryopreservation protocol for the human SMDC cell-microcarrier combination.

Methods

Human SMDC from five different donors were attached to the surface of implantable microcarriers composed of poly(DL-lactide-co-glycolide) in mini bioreactors. The cellularized microcarriers were then incubated with two different types of cryoprotective agent (CPA) [CryoStor10® (CS10) containing 10% Dimethylsulfoxide or CS10Gr (CS10 containing 10% GranuGel®, a colloidal hydrogel)]. The samples were subjected to slow freezing (-1^{o}C/min) using a controlled rate freezer and stored at liquid nitrogen for at least a week. The samples were thawed in either air (slow) or water (rapid) at 37^{o}C followed by *in vitro* culture for 0hr or 24hrs. Cells from the samples were characterized in terms of cell viability, adhesion and myogenic

differentiation (fusion index). Cellularized microcarriers that had not been cryopreserved were used as the control group.

Results

Cell viability: Cellularized microcarriers thawed rapidly resulted in cell viability ≥74% at 0hr post-thawing and ≥62% at 24hrs post-thawing. Samples thawed slowly had reduced cell viability. Cell number: After thawing, the quantity of cells attached to the microcarriers was decreased compared with before cryopreservation. Samples cryopreserved in CS10Gr and thawed slowly had fewer cells than the samples rapidly thawed. Cell phenotype: Except for the samples cryopreserved in CS10Gr and thawed slowly, cell differentiation after cryopreservation was equivalent with cells that had not been cryopreserved.

Conclusions

Our study indicates cryopreservation of the implantable cell-microcarrier combination is technically feasible. Most cells remain attached to the microcarriers following cryopreservation and thawing and the capacity of human SMDC to differentiate into myotubes is unaffected. These findings suggest use of cold-chain product supply can be used for future clinical studies with the implantable cell-microcarrier technology.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 874807.

Topic: Translation

Subtopic: Cell therapies

Type: Poster presentation

TERMIS25_81 Bio-functionalized hydrogel with Schwann cells alleviated inflammation and triggered axonal sprouting after spinal cord injury. Yashar Mehrbani Azar[1], Liudmila N. Novikova[1], Vijay Kumar Kuna[1], Maria Vittoria Giraudo[1], Mathias Selin[1], Mikael Wiberg[2], Lev N. Novikov[1]

[1]Department of Medical and Translational Biology, Umeå University, Umeå, Sweden; *[2]Department of Medical and Translational Biology, Umeå University, Department of Surgical and Perioperative Science, Section of Hand and Plastic Surgery, Umeå University, Umeå, Sweden*

Introduction/Objectives

Injuries to the spinal cord (SCI) affect thousands of economically active, often young, people of both genders worldwide every year. SCI is always associated with nerve tissue degeneration, neuroinflammation, and the development of cysts and cavities surrounded by astrocytic and fibrotic scar tissue.

Methods

In this study, we evaluated the effects of a biodegradable nerve tissue-derived hydrogel combined with cultured adult rat Schwann cells on astrocyte and microglial cell reactivity *in vitro*, as well as on axonal sprouting and glial cell responses following C3-C4 cervical spinal cord injury in adult rats.

Results

In vitro studies demonstrated that the hydrogel, either alone or in combination with cultured Schwann cells, down-regulated the expression of several growth-inhibitory chondroitin sulfate proteoglycans in astrocytes and the cytokine TNFα in primary microglial cell cultures. Data from a Proteome Profiler Rat XL Cytokine Array demonstrated that the tested hydrogel not only maintained but also induced the secretion of new growth-promoting cytokines and chemokines in both non-reactive and activated astrocytes.

Following implantation into the injured spinal cord, the hydrogel supported the survival of transplanted GFP-labeled Schwann cells and significantly decreased the density of reactive astrocytes and microglial cells. Additionally, the hydrogel, either alone or in combination with Schwann cells, induced sprouting of serotonergic (5HT) axons in the ventral horns, rostral and caudal (C2 and C6 spinal segments) to the injury

site. Moreover, the treatment reduced aberrant sensory sprouting from CGRP-positive axons in lamina III of the C6 cervical dorsal horn.

Conclusions

These results suggest that nerve tissue-derived hydrogel with Schwann cells can support regeneration after SCI.

Topic: Translation

Subtopic: Cell therapies

Type: Poster presentation

TERMIS25_676 Microcarrier-based stirred culture systems for the development of tissue engineered products towards muscle repair. Cláudia Lobato da Silva[1 2], Teresa Franchi-Mendes, Ana Luísa Cartaxo[1 2], Ana Fernandes-Platzgummer[1 2], Carlos Av Rodrigues[1 2], Karin Jöhrer[3], Richard Day, Joaquim Ms Cabral[1 2]

[1]Department of Bioengineering and iBB - Institute for Bioengineering and Biosciences at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; *[2]Associate Laboratory i4HB - Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal*; *[3]Innovacell Biotechnologie AG, Mitterweg 24, A-6020, Innsbruck, Austria*

Introduction/Objectives

Therapeutic approaches for dysfunctional sphincters are essential for patients suffering from fecal incontinence, which affects about 10% of the European population, though this value may be underestimated due to social stigma associated. Current treatment modalities are limited, therefore cell therapy has gained momentum in the field of incontinence management. However, challenges remain concerning cell manufacturing and establishing objective measures of therapeutic success.

This work comprises two main aims: the first focuses on establishing a GMP-compliant cell-microcarrier product containing skeletal-muscle derived cells (SDMC) and biodegradable microcarriers; and the second addresses the development of culture platforms using commercially available microcarriers for the scalable expansion SDMC, with a comparison between Vertical Wheel bioreactors and spinner flasks systems.

Methods

For the first aim, SDMC were cultured under planar conditions, followed by transfer to Vertical Wheel bioreactors to be combined with biocompatible and biodegradable poly(lactic-co-glycolic) (PLGA) microcarriers, previously prepared by thermally induced phase separation (TIPS). Concerning the second main aim, focusing on the expansion in microcarrier-based systems, three types of commercial microcarriers were screened and Dissolvable microcarriers (from Corning) were selected for further studies. Two stirred-systems were tested for expansion of SDMC under xeno(geneic)-free conditions: single-use Vertical wheel bioreactors (PBS Biotech) and spinner flasks. Importantly, for both aims, SMDC identity was validated by flow cytometry and a myotube formation assay.

Results

To achieve an implantable cell-microcarrier product, under optimized stirred conditions, 70-80% SDMC adhesion onto PLGA microcarriers was obtained with high viability. In parallel, two platforms were tested for cell manufacturing: Vertical wheel bioreactor and spinner flask. Three types of microcarriers were screened and only Dissolvable microcarriers supported expansion of SDMC in stirred conditions. After, SDMC from 3 donors were tested in both stirred systems, using culture medium supplemented with a gamma-irradiated human platelet lysate and fibroblast growth factor-2. After 6 days, both systems achieved similar cell expansion levels, 8-10-fold, with no statistically significant differences observed. Importantly, cell phenotype was validated by detection of CD56 and by performing a close to function readout based on the myogenic differentiation potential into myotubes. No differences were found between the cells expanded

in spinner flask or those in Vertical wheel bioreactors; the only observed variability was between individual donors.

Conclusions

The xeno-free conditions combined with the single-use feature of the bioreactor make this platform GMP compliant. The use of implantable microcarriers should increase the likelihood of success of the potential therapy, as it avoids the drawbacks associated with SDMC harvesting and subsequent administration as cell suspension. This cell-microcarrier product will be tested in a phase I/II clinical trial under the scope of the AMELIE consortium (www.amelie-project.eu). Concerning cell manufacturing, our results suggest the feasibility of two scalable platforms for expanding human SMDC, envisaging easy-to-implement biomanufacturing processes in a time- and cost-effective way.

Topic: Translation

Subtopic: Cell therapies

Type: Poster presentation

TERMIS25_1278 Autologous fibroblasts: isolation, cultivation, and application technique. Volker Eras[1], Thilo Brill[1], Norus Ahmed[1], Jan Brune[1]

[1]*German Institute for Cell and Tissue Replacement (DIZG, non-profit GmbH), Berlin, Germany*

Introduction/Objectives

Fibroblasts are essential cellular components in wound healing, particularly in burn treatment. These versatile cells play a crucial role throughout the entire wound healing process, from the late inflammatory phase to the final epithelialization of the injured tissue[1]. Their multifaceted functions make them indispensable for effective tissue repair and regeneration. Given the critical importance of fibroblasts in wound healing, it is imperative to understand these cells for optimal therapeutic applications. To this end, the primary objectives of this work are to isolate fibroblasts, assess their identity and quality, and evaluate the safety of both the cells themselves and the comprehensive processing procedure.

Methods

The study evaluates the viability, growth, and morphology of fibroblasts at different stages: isolation, cultivation, and post-spray application. A standard wound closure assay, utilizing Collagen I staining, was employed to confirm cell identity. Additionally, four different syringes were tested to assess their effects on cell yield and viability following spraying.

Results

Autologous dermal fibroblasts were successfully isolated from skin biopsies from which autologous keratinocytes were obtained prior to this. Fibroblast yields varied among patients. The isolated cells exhibited high viability and the characteristic elongated fibroblast phenotype, aligning in parallel as confluency increased. Initial seeding of approximately 9,000 cells resulted in final densities between 20,000 and 150,000 cells per cm^2. Doubling times and overall cell numbers demonstrated high patient dependency. Optimized spray application methods using different syringes and shaking improved initial low cell yields without compromising viability. Post-spray cultivation showed continued growth, maintaining patient-specific patterns observed during initial cultivation with no cytotoxic effects noted. In a standard in vitro wound closure assay, fibroblasts began migrating into a 500 µm gap within 24 hours, achieving complete gap closure after 3 days, as evidenced by Collagen I expression. The cultured fibroblasts met quality and safety standards, showing no contamination and maintaining viability throughout the process.

Conclusions

The study successfully demonstrates the effective isolation and cultivation of autologous dermal fibroblasts from skin biopsies. The results indicate that these fibroblasts maintain high viability and characteristic morphology throughout the isolation and more importantly after the clinical application

processes. The ability of sprayed fibroblasts to migrate and close wounds underscores their potential therapeutic value in regenerative medicine. Overall, this work provides necessary proof in delivering functional fibroblasts in a clinical setting that meets regulatory measures. Hence, the clinical use of autologous fibroblasts is now available to German burn patients.

1. Ter Horst B, Chouhan G, Moiemen NS, Grover LM. Advances in keratinocyte delivery in burn wound care. Advanced drug delivery reviews. 2018 Jan 1;123:18-32.

Topic: Translation

Subtopic: Clinical application of cell-based constructs

Type: Poster presentation

TERMIS25_995 Tissue engineered blood vessels.

Nevena Slavova[1]

[1]*University of Sheffield, Kroto Research Institute, University of Manchester, Sheffield, United Kingdom*

Introduction/Objectives

Cardiovascular disease (CVD) is the most common cause of death worldwide, with disease incidence increasing annually. CVD is often a result of atherosclerosis leading to vessels stenosis. The current treatment is a coronary bypass surgery where the occluded vessel is bypassed using an autologous saphenous vein or synthetic graft (e.g PTFE, Dacron) to revascularize the region. However, autologous grafting is associated with, poor quality, lack of availability and donor site morbidity. Synthetic grafts are routinely used for large vessel grafting but poor patency and thrombosis, limit their use for small diameter applications (<6mm). To address these limitations, we propose a tissue engineered vascular graft (TEVG) that harnesses the properties of porous polymers and the robust ability of fibroblasts to produce extracellular matrix, particularly collagen and elastin. Macromolecular crowding (MMC), mechanical stimulation and bioreactor flow regiments are being explored to enhance collagen and elastin production. The mature graft will be decellularised with the final aim being a non-immunogenic and universal graft that matches the burst pressure, elasticity and suturability of native arteries.

Methods

The polymer scaffolds were manufactured via emulsion templating of poly(glycerol sebacate)-methacrylate (PGS-M) to produce photocurable, high internal phase emulsions (polyHIPEs). The PGS prepolymer is a result of a polycondensation reaction between glycerol and sebacic acid, which is functionalised with methacrylic anhydride, to become photocurable. The polyHIPE scaffolds were created by adding water (internal phase) to PGS-M polymer (external phase) with the use of emulsion stabilising surfactant. Two types of scaffolds were produced – discs for optimisation and tubular for the TEVG. Immortalised human fibroblasts, BJ-5TA were cultured on the disc scaffolds. MMC of cell culture media with carrageenan and polyvinylpyrrolidone is being optimised to increase collagen/elastin production.

Results

Scanning electron microscopy analysis was used to select the polyHIPE composition with a median pore size of 30 microns ranging between 10-100 microns. The selection was also based on the higher cell metabolic activity experienced by fibroblasts cultured on scaffolds formulated with 5% surfactant compared to scaffolds formulated with 10% surfactant. Cells readily infiltrate the selected PGS-M discs, demonstrated by histological analysis. Tubular scaffolds with internal diameter of 3.8 mm were successfully manufactured using the same polyHIPE composition. Interestingly, preliminary data from crowding experiments shows a decreased collagen production by the BJ-5TAs, contradicting trends with other cell types described in literature.

Conclusions

The selected polyHIPE allows for efficient cell infiltration and is suitable for creating 3.8 mm internal diameter tubular scaffolds. MMC experiments suggest that carrageenan may not be a suitable crowding

agent for immortalised fibroblasts and polyvinylpyrrolidone is being explored as an alternative to increase collagen and elastin production. Future work will also focus on optimising rotational seeding techniques for efficient and uniform distribution of cells within the tubular scaffolds. Suitable decellularisation methods will be evaluated with the main criteria being retention of extracellular matrix proteins and efficient removal of antigenic material.

Topic: Translation

Subtopic: Clinical regenerative medicine

Type: Poster presentation

TERMIS25_217 Extrusion-based 3D bioprinting from bench to bedside applications in bone tissue engineering using platelet rich plasma and scaffolding materials. Dongxu Ke[1], Xi Yang[1]

[1]*Novaprint Therapeutics, Suzhou, China*

Introduction/Objectives

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 teams, patients' specific implants were printed in a GMP lab with an ISO 5 environment. The clinical trials were conducted using these patients' specific implants in the bone defects after removing tumors.

Results

The printed constructs showed a very low level of endotoxin with zero occurrences of contamination after the printing. Meanwhile, results showed excellent osteointegration and osteoinduction between implants and host tissues. All patients could release from the hospital after one week of observation in the hospital without any complications.

Conclusions

This bench to bedside application of 3D bioprinting and regenerative medicine is promising and should be widely promoted in more hospitals.

Topic: Translation

Subtopic: Clinical regenerative medicine

Type: Poster presentation

TERMIS25_954 Towards translation of bone tissue engineering: Assessing novel biodegradable microcarriers for the expansion and differentiation of mesenchymal stromal cells. Tess Uphof[1], Maik Schot[2], Tom Kamperman[2], Ruud Das[3], Eric Farrell[1]

[1]*Erasmus Medical Center, Rotterdam, Netherlands*; [2]*IamFluidics B.V., Enschede, Netherlands*; [3]*Scinus Cell Expansion Netherlands B.V., Zeist, Netherlands*

Objectives

In order to translate mesenchymal stromal cell-based (MSC) bone tissue engineering to the clinic, MSC culture must be scaled up in a cost-effective, automated and GMP-compliant manner. Therefore, we aim to develop a system in which MSCs can be expanded and differentiated on biodegradable microcarriers in an enclosed bioreactor system. We have generated gelatine-based microcarriers with different compositions, thereby expected to have altered degradation rates, to find a suitable carrier for bone formation via

endochondral ossification. The carriers were tested for homogeneity regarding size and shape and their ability to support MSC expansion and differentiation without the need to detach the cells from the carriers.

Methods

Microcarriers were generated using an in-air microfluidic platform in 3 different formulations with differing gelatine contents (IamFluidics B.V). Size distribution and circularity were determined using microscopy and digital image analysis. 200.000 human paediatric MSCs were seeded in a spinner flask containing 250 cm^2 microcarriers in 50 mL GMP-compliant medium (αMEM, 2.5%Human Platelet Lysate, 50µg/mL gentamycin, 1.5µg/mL fungizone, 0.1mM ascorbic acid, 1ng/mL bFGF). Cells were visually inspected and counted over time. When reaching a distribution of at least 30%, cultures were expanded to 1250 cm^2 in the same volume. Cell viability was determined by a live/dead stain (Calcein-AM/PI). When reaching a confluency of 70-90%, samples were switched to chondrogenic differentiation medium (DMEM-HG+Glutamax, 50µg/mL gentamycin, 1.5µg/mL fungizone, 1% ITS, 40 ug/mL proline, 1mM sodium pyruvate, 0.1mM ascorbic acid, 10ng/mL TGFb3, 100nM dexamethasone) in a static or dynamic environment. Cell pellets from monolayer expanded cells were taken as a positive control. After 3 weeks, chondrogenesis was assessed by histology and gene expression.

Results

The generated microcarriers were circular and monodisperse, as evidenced by a minimum circularity of 84% and a coefficient of variation in particle diameter of ≤7.1% per batch. The three carriers had an average diameter of 201 µm, 142 µm and 169 µm. After seeding, adequate cell attachment was observed for all carrier types. During expansion, population growth and a high cell distribution over the carriers of near 100% was seen for all carrier types, confirming bead-to-bead transfer occurred. Furthermore, attached cells were proven to be viable on all microcarriers after 7 days of culture. Within 9-10 days, carriers reached a confluency of 70-90% and samples for chondrogenic differentiation were prepared. Static microcarrier samples showed an upregulation of chondrogenic markers COL2A1 and COL10A1, comparable to that of monolayer-expanded cell pellets. However, for the dynamic samples this expression was much lower.

Conclusions

Three monodisperse gelatine-based microcarriers were generated with varying gelatin-contents, expected to give rise to altered degradation rates *in vivo*. MSCs exhibited good attachment, uniform distribution over the carriers and high viability during the culture period. Furthermore, cells were successfully, chondrogenically differentiated directly on the microcarriers in a static setting. However, performing a dynamic differentiation hampered this process. Future research will focus on improving this dynamic differentiation and assessing the most suitable microcarrier with the best degradation rate to accompany bone formation *in vivo*.

Topic: Translation

Subtopic: Commercialisation

Type: Poster presentation

TERMIS25_623 Empowering Women Entrepreneurs in Bio&MedTech: Innovative Strategies for Overcoming Barriers and Achieving Work-Life Balance.

jovanna cervantes guzman[1], Shane Mathews[2] [3], Dietmar Hutmacher[4] [5] [6]

[1]QUT Business School, The Australian Centre for Entrepreneurship Research, Queensland 5 University of Technology, Brisbane, Australia, 2 6 Centre for Biomedical Technologies, School of Mechanical, Medical and Process 7 Engineering, Queensland University of Technology, Brisbane, QLD 4000, Australia, 3 ARC Training Centre for Cell and Tissue Engineering Technologies, Queensland University 9 of Technology, Brisbane, QLD 4000, Australia, Brisbane, Australia; *[2]QUT Business School, The Australian Centre for Entrepreneurship Research, Queensland 5 University of Technology, Brisbane, Australia*; *[3]School of*

Advertising, Marketing and Public Relations, QUT Business School Queensland University of Technology, Brisbane, Australia; [4]*QUT Business School, The Australian Centre for Entrepreneurship Research, Queensland 5 University of Technology, Centre for Behavioural Economics, Society and Technology (BEST)Queensland 13 University of Technology, 6Max Planck Queensland Centre (MPCQ) for the Materials Science of Extracellular Matrices, 15 Queensland University of Technology, Brisbane, Australia*; [5]*Centre for Biomedical Technologies, School of Mechanical, Medical and Process 7 Engineering, Queensland University of Technology, Brisbane, Australia*; [6]*ARC Training Centre for Cell and Tissue Engineering Technologies, Queensland University 9 of Technology, Brisbane, Australia*

Introduction/Objectives

Introduction: The Bio&MedTech industry holds immense potential for innovation, yet women entrepreneurs in this field face significant barriers. This study focuses on understanding the work-life balance strategies of successful women entrepreneurs from Australia, Europe, the USA, Canada, and Latin America, highlighting the multidimensional challenges they encounter and the solutions they employ.

Despite the growing presence of women in Bio&MedTech, gender-specific barriers persist, hindering their entrepreneurial intentions and success. Limited access to funding, inadequate networks, and gender bias contribute to these challenges. This study investigates how these women navigate and overcome such obstacles.

Objective: This research aims to explore and analyze the strategies employed by women entrepreneurs in Bio&MedTech to achieve a work-life balance, contributing to the journal's themes of multidisciplinarity and innovation for sustainable development. It aligns with the OECD Oslo Manual's guidelines and the United Nations 2030 Agenda for Sustainable Development Goals.

Methods

The study used a qualitative, exploratory research design with semi-structured interviews conducted with 21 women entrepreneurs. Data was collected through in-depth interviews, transcribed verbatim, and analyzed using thematic analysis with the support of NVivo software. The reliability and validity of the data were ensured through triangulation and member checking.

Results

The research identified key strategies such as mentorship, networking, and leveraging unique capabilities like emotional intelligence and resilience. These strategies help women entrepreneurs overcome barriers and achieve success in the Bio&MedTech industry, promoting innovation for sustainable development.

Conclusions

The study concludes that targeted strategies significantly aid women entrepreneurs in overcoming barriers and achieving success. Limitations include the small sample size and regional focus, suggesting future research should expand to diverse contexts and larger populations. The findings underscore the importance of multidisciplinarity and innovation for sustainable development.

Topic: Submit to SYMPOSIUM

Subtopic: Recent advances in articular cartilage engineering

Type: Poster presentation

TERMIS25_422 Development and evaluation of an in vitro osteoarthritis model using scaffold-free cartilage-like microtissues. Annemarie Neubert[1], Saharnaz Hashemi[1], Christian Schmidt[2], Ursula Anderer[1]

[1]*Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology, Cell Biology and Tissue Engineering, Universitaetsplatz 1, 01968, Senftenberg, Germany*; [2]*Sana Klinikum Niederlausitz, Department Trauma Surgery and Orthopedics, Senftenberg, Germany*

Introduction/Objectives

Osteoarthritis (OA) is a widespread disease affecting over 500 million people worldwide. There is currently no cure, only treatment of the symptoms. Various *in vivo* and *in vitro* models have been developed to gain better insight into the disease. However, those models are either animal-based or utilise scaffolds. The aim of this study was to establish an *in vitro* model for osteoarthritis using scaffold-free cartilage-like microtissues. Furthermore, the use of this model as pharmacological test system should be evaluated.

Methods

Human articular chondrocytes were isolated from a condyle of a 64-year old male patient and expanded as monolayer. Scaffold-free microtissues were generated and cultivated using u-repellent plates. After four weeks cultivation, microtissues were treated for one week with IL-1β to induce osteoarthritis. The following week, IL-1β-treated microtissues were subjected to different therapies including naproxen and TGF-β_3 with and without the presence of IL-1β. Untreated microtissues served as control at all time points. The macroscopic appearance was documented via reflected light microscopy and the size determined. The differentiation degree was evaluated via histology and immunohistology (IH). Furthermore, the metabolic activity of microtissues was determined via quantification of the ATP content and the viability of the cells within the microtissues via propidium iodide (PI) staining. Additionally, the glycosaminoglycan (GAG) content using the DMMB assay as well as the wet weight were assessed.

Results

All microtissues were white in colour and had a similar size. There was no difference in the histological analyses of glycosaminoglycans or the expression of Sox9. The treatment of IL-1β led to a reduction of ATP and GAG content by 50% as well as a reduction in wet weight. Dead cells were present within the microtissues evidenced by PI. Furthermore, IH analyses revealed a reduction in collagen type II and proteoglycans as well as a slight increase in collagen type I. The therapies with naproxen and TGF-β_3 could partially increase the wet weight, the ATP content as well as collagen type II. Furthermore, the amount of dead cells present was also reduced.

Conclusions

This study shows that it is possible to introduce an osteoarthritis-like condition in cartilage-like microtissues by the treatment with IL-1β. The *in vitro* therapy with naproxen and TGF-β_3 proofs the applicability of this *in vitro* disease model as pharmacological test system. This model could potentially help in elucidating the mechanisms of OA as well as finding potential therapeutic agents. Moreover, the model could be expanded by adding other tissues, e.g. bone-like microtissues, to mimic the articular joint space.

Topic: Translation

Subtopic: Drug screening models

Type: Poster presentation

TERMIS25_1435 Optimisation of Printed Microfluidic Devices as Preclinical Drug Screening Platforms. Francisca Barbosa[1], David Patrocínio[2], Juan Carlos Gómez-Blanco[2], José Blas[2], Paula Coutinho[1], Sonia Miguel[1]

[1]BRIDGES - Polytechnic University of Guarda, Guarda, Portugal, Guarda, Portugal; *[2]Centro de Cirugía de Mínima Invasión Jesús Usón, Cáceres, Spain*

Introduction/Objectives

The production of microfluidic systems is an emerging area of research in the development of the preclinical platforms for in vitro drug screening, which is an excellent strategy in line with the 3Ŕs policies. Such platforms will help reduce the number of animals used in scientific experimentation, thereby minimizing ethical concerns and the time associated to the drug commercialization process.

Considering this, the 3D printing arises as an attractive technique to be used in the production of microfluidic systems, due to its ability to construct structures with a predefined size and geometry. However,

it is necessary to ensure that the cell interaction with printed surfaces is promoted. Therefore, in this work, different materials and parameters were tested by using the stereolithography technique aiming to validate its implementation in the production of microfluidic systems. Subsequently, the coating of printed surfaces with bioadhesive materials was also assessed by evaluating the impact on cell interaction, and proliferation.

Methods

The microfluidic devices were printed by using resins and exploring two different 3D Printers. In addition, the microfluidic surfaces were coated with chitosan, gelatin and lysine was conducted through solvent evaporation method. To evaluate the effect of bioadhesive coatings on cell viability and proliferation, crystal violet and fluorescence microscopy were performed.

Results

In general, it was verified that the type of resin used in the printing process was found to have a direct influence on the pattern and roughness of chips' surface. On the other hand, the coating of microfluidic devices effectively contributed to improve the cell behaviour of the human fibroblasts and epithelial cells. The cell viability and adhesion were prompted on coated microfluidic devices.

Conclusions

This work has demonstrated the importance of functionalizing microfluidic systems' surface printed by stereolithography with bioadhesive materials to enable cell culture within these systems, and to further mimic the native composition of the tissues. In the future, more detailed characterization of the microfluidics' surface and evaluation of the cell behaviour of other cell types will be paramount to validate the stereolithography printed microfluidic devices as promising preclinical platforms for in vitro drug screening.

Topic: Translation

Subtopic: Personalized medicine applications

Type: Poster presentation

TERMIS25_723 Optimizing the regenerative potential of vaginal fibroblasts: the role of autologous platelet-rich plasma and hyaluronic acid in vitro. Sarah Berndt[1], Solange Vischer[1], Antoine Turzi[2], Ali Modarressi[3], Patrick Dallenbach[4]

[1]Regen Lab SA, 1052 Le Mont-sur-Lausanne, Switzerland, Switzerland; *[2]RegenLab SA, Le Mont sur Lausanne, Switzerland*; *[3]Hirslanden CH, Geneva, Switzerland*; *[4]Geneva University Hospitals, Geneva, Switzerland*

Introduction/Objectives

Platelet-rich plasma (PRP) is an autologous blood product used to stimulate regeneration, while hyaluronic acid (HA) supports tissue hydration. This study examines the in vitro effects of PRP alone or combined with HA on vaginal fibroblasts (VF) isolated from mucosal samples, aiming to improve aging and atrophic vaginal tissue.

Methods

Vaginal and blood samples were collected from postmenopausal women undergoing pelvic organ prolapse surgery. PRP was prepared alone or combined with HA (PRP-HA) from peripheral blood. Vaginal fibroblasts were isolated via enzymatic dissociation and cultured in 2D and 3D (spheroids) systems. Biological activities were assessed by measuring cell growth, proliferation, senescence, metabolic activity, and collagen accumulation, along with immunocytochemistry for (myo)-fibroblast markers.

Results

VFs cultured with PRP or PRP-HA showed dose-dependent higher proliferation compared to fetal bovine serum controls, with increased S and G2M cell cycle phases correlating with enhanced proliferation. Vimentin expression was stable, while alpha-SMA decreased, indicating a shift from myofibroblasts to

fibroblasts. Collagen accumulation increased under PRP or PRP-HA treatment, crucial for wound healing. PRP and PRP-HA also prevented cell senescence during long-term low-density cultures. These findings were consistent in both 2D and 3D culture systems.

Conclusions

This study provides in vitro evidence supporting the potential of PRP and PRP-HA as autologous treatments for vaginal rejuvenation.

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_300 The contribution of novel genes to fracture risk in Thoroughbred horses. Debbie Guest[1], Amy Ross[1], Ellison Lumsden[1]

[1]*Royal Veterinary College, Hatfield, United Kingdom*

Introduction/Objectives

Bone fractures often occur in Thoroughbred racehorses due to high-intensity, repetitive loading that over time leads to structural failure. Fracture risk is affected by genetic and environmental factors. We previously established induced pluripotent stem cells (iPSCs) from horses at high and low genetic risk and differentiated them into bone forming osteoblasts. RNA sequencing revealed 112 differentially expressed genes (DEGs). Of these 27 were not annotated, 43 had a published role in bone and 42 had no published role in bone. The objective of this study was to determine the role of these novel genes in osteoblasts.

Methods

We first screened the expression of all 42 of the DEGs with no published role in bone and four of the DEGs with a published role in bone (*CHAC1*, *LGALS1*, *NR2F1*, *NR2F2*) in Saos2 cells cultured under basal proliferative conditions and after 21 days of osteogenic culture. All four of the genes with a published role in bone and 26 of the 42 genes with no published role in bone were expressed under basal and/or osteogenic conditions.

We then modulated the expression of seven of these genes (*ADSSL1*, *CABP1*, *ENO2*, *KIZ*, *LGALS1*, *SPARCL1* and *UCP2*) in Saos2 cells via stable overexpression or gene knockdown using a short hairpin RNA. Effects on cell viability, gene expression and matrix deposition were measured.

Results

LGALS1 overexpression resulted in a 17% decrease in cell viability in basal conditions ($p<0.05$) and decreased mineralisation by 90% under osteogenic conditions ($p<0.01$). However, it had minimal differences on alkaline phosphatase (ALP) activity or gene expression under either basal or osteogenic conditions.

LGALS1 knockdown significantly reduced cell viability by 53% in basal conditions ($p<0.001$), increased matrix mineralization by 95% ($p<0.001$) and increased ALP activity by 64% after 7 days of osteogenic culture ($p<0.01$). There were also significant increases in *MEPE*, *SPP1*, *SP7*, *PHEX* and *SOST* expression under basal conditions. After 21 days of osteogenic culture there were significant increases in *MEPE, ENPP1* and *RUNX2* and significant decreases in *COL1A1*, *DMP1* and *SP7* ($p<0.05$).

We were unable to modulate *KIZ* expression, supporting previous evidence that it may be part of an essential pathway in Saos2 cells. While we were successfully able to overexpress *SPARCL1*, we were unable to knock it down suggesting that it may also be an essential gene. We were able to successfully modulate the expression of *ADSSL1*, *CABP1*, *ENO2*, and *UCP2.* Overexpression of *ADDSL1*, *CABP1*, *SPARCL1* and *UCP2* all significantly reduced cell viability by around 80% ($p<0.05$). Work to determine the effects on matrix mineralisation and gene expression is ongoing.

Conclusions

The modulation of genes that are differentially expressed in iPSC-osteoblasts derived from horses at high and low genetic risk of fracture has significant effects on the viability, gene expression and matrix mineralisation of bone forming Soas2 cells. This suggests that their mis-expression may be directly contributing to fracture risk. A deeper understanding of the biological processes that are affected in genetically high-risk horses may enable the future development of novel interventions to reduce fracture risk.

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_389 Equine induced versus primary mesenchymal stem cells: which is the winning horse?. Belén Serrano Pastor[1], Elvira Bernad Roche[1], Alina Cequier Soler[1 2], Francisco José Vázquez Bringas[1 2], Antonio Romero Lasheras[1 2], Pilar Zaragoza Fernández[1], Arantza Vitoria Moraiz[1 2], Clementina Rodellar Penella[1], Laura Barrachina Porcar[1 2]

[1]Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza); Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA); Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain; *[2]Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain*

Introduction/Objectives

The advancement of equine regenerative medicine requires optimising the use of biological therapies, such as stem cells. Tissue-derived (primary) mesenchymal stem/stromal cells (MSCs) have shown promising results but have certain disadvantages. The differentiation potential of MSCs is restricted to the mesodermal lineage, and they enter senescence during *in vitro* culture. This implies the need of repeatedly obtaining tissue to isolate new MSCs, increasing the heterogeneity of the cell product. To overcome these limitations, work has recently begun on another stem cell type: induced pluripotent stem cells (iPSCs). These cells can virtually differentiate into any cell type and have been proposed as an alternative source of MSCs: induced MSCs (iMSCs). The use of iMSCs could potentially eliminate the need for repeated tissue harvesting, increase cell production, and improve the homogeneity of the product. However, while iMSCs are quickly raising interest in human medicine, very little is known about these cells in the horse. Since iMSCs derive from genetically reprogrammed cells, their similarity and potential advantages over primary MSCs need to be clarified by studying their behaviour and functional characteristics.

The aim of this study is to compare iMSCs with primary MSCs in terms of standard characterisation and *in vitro* expansion profile, to determine whether iMSCs can be advantageous in the equine species.

Methods

Equine iMSCs were derived from iPSCs via the neural crest pathway, and primary MSCs were obtained from bone marrow (BM-MSCs). To study the immunophenotype, the following surface markers were analysed by flow cytometry: CD105, CD90, CD44, CD11α/18, CD45, MHC-I and MHC-II. Tri-lineage differentiation was assessed using specific differentiation media. Proliferation and growth kinetics was studied by calculating their doubling time, by performing MTT assays to measure cell metabolic activity, and by flow cytometry to analyse the cell cycle and apoptosis (annexin V assay). To compare the senescence profile of both cell types, a beta-galactosidase assay was performed. In addition, different genes related to apoptosis (*BAX*, *BCL-2*, *BCL-XL*, *CASP-8* and *HSP-27*) and proliferation (*COX1* and *Cyclin D2*) were analysed by RT-qPCR.

Results

We observed immunophenotype and tri-lineage potential differences between iMSCs and BM-MSCs, with inter-donor/inter-line variability in both cell types. Regarding the immunophenotype, negative markers

were overall not expressed in either MSC type, but positive markers showed more variable expression. For the tri-lineage differentiation, adipogenesis was complete and similar in both MSC types, but osteogenic and chondrogeneic potentials were lower for iMSCs.

Differences were also observed between the growth kinetics of iMSCs and BM-MSCs. iMSC proliferation was slower at earlier passages but their growth rate increased along the culture time, while proliferation decreased in primary MSCs over passages. These observations may be related to different patterns of apoptosis and senescence between the cell types.

Conclusions

Equine iMSCs can be a suitable alternative to primary MSCs, reducing tissue collection and potentially yielding higher cell numbers with less heterogeneity. More studies are needed to functionally characterize iMSC and to explore their safety and therapeutic potential over primary MSCs, with particular attention to their immunomodulatory and immunogenic profiles.

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_613 Procedures for scaffold reconstruction of tendon: an experimental study.

Alberto crovace[1], Luca Lacitignola[2], Riccardo Giorgino[2], Marta Guadalupi[2], Alberto Sensini[3], Luca Cristofolini[3], Maria Letizia Focarete[3], Eraldo Sanna Passino[4], Francesco Staffieri[2], Antonio Crovace[2]

[1]Dipartimento di Medicina Veterinaria Università di Sassari, sassari, Italy; *[2]DIMEPRE-J University of Bari, Bari, Italy*; *[3]Università di Bologna, Bologna, Italy*; *[4]Uniss, Sassari, Italy*

The occurrence of tendon injuries/defects caused by trauma, age-related degeneration, or excessive loading of the musculoskeletal system is a common clinical problem in Human and Veterinary patients. Currently, the conventional clinical treatments include immobilization, physiochemical therapy, and surgical suturing. Although suture surgery provides temporary restoration of tendon continuity, it fails to retain the structural integrity and mechanical strength. Biomaterials have been demonstrated to promote the regeneration and the treatment of tendon injuries with some advantages such as morphological restoration, unrestricted material source, excellent biocompatibility and absence of immune reactions. Various fabrication methods have been developed such as 3D bioprinting, wet-spinning, and electrospinning. Our group had the possibility to validate an electrospinning bioabsorbable scaffold obtained from a new technology that uses an electrically-driven method to produce fibers of nanometric or micrometric diameter that can reproduce the hierarchical structure and the mechanical propriety of tendinous and ligamentous tissues in the Achilles tendon of an ovine model. The aim of the study, authorized by the Italian Ministry of Health (n° 733/2023-PR), was to find a surgical system that could guarantee the tightness of the tendon itself, even in the presence of a large loss of substance, which had to be left free to carry out its normal functions during the tendon regeneration. In this study we report the surgical employed techniques and the first results. 12 sheep were trichotomized and underwent premedication and subarachnoid anesthesia in preparation for surgery. A longitudinal skin incision was made corresponding to the Achilles tendon of approximately 7 cm and then the tendon was isolated. The common tendon sheath that envelops the two terminal heads of the tendons was incised allowing the two ends to be highlighted and isolated with a mosquito forceps placed between them. At the two ends of the sheath incision, two full-thickness n.2 nylon sutures were applied to join the two tendon heads together. At this point two technique of implant of scaffold were applied:

1) a medial portion of 4 cm x 5 mm of Gastrocnemius tendon was cut and removed and replaced with a scaffold of the same measure that was sutured proximally and distally to the excised tendon with a Bunnell

suture in nylon 3.0 monofilament and laterally with a monofilament of PDS 3.0. Then the tendon sheath was sutured with a reabsorbable monofilament 3.0.

2) after the incision of the common tendon sheath and the application at the two ends of the sheath incision of the two full-thickness nylon n.2 sutures, the Gastrocnemius tendon was isolated and a partial longitudinal incision was made to get a pocket to receive the scaffold.

The tendon incision and the common tendon sheath was sutured with a 3.0 monofilament reabsorbable suture. In the two techniques the subcutaneous tissue was sutured with a reabsorbable monofilament 3.0 suture and the skin was closed with staples. Clinical and ultrasound checks were performed in the post operative period to check the mechanical tightness of the systems and evaluate the regenerative and mechanical capacity of the scaffold.

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_822 Optimisation of three-dimensional muscle scaffold: fiber clearance for new cell integration. Rossana Topputi[1], Eylem Emek Akyurek[2], Anna Carolo[2], Luca Melotti[2], Martina Erba[2], Gianluca Ventriglia[1], Marco Vincenzo Patruno[2], Roberta Sacchetto[2], Tiziana Martinello[1]

[1]Department of Veterinary Medicine, University of Bari Aldo Moro, Italy, valenzano (BA), Italy; *[2]Department of Comparative Biomedicine and Food Science, University of Padua, Italy, Legnaro (PD), Italy*

Introduction/Objectives

Three-dimensional (3D) muscle organoids represent an advanced in vitro platform that can emulate functional muscle tissue, addressing limitations of traditional monolayer cultures and minimizing the need for animal models in research. For skeletal muscle organoids, the inclusion of an extracellular matrix (ECM) scaffold is essential to achieve structural and functional fidelity, making decellularized tissue a preferred scaffold choice. Our previous work introduced a muscle organoid derived from the dorsal portion of bovine diaphragm, a unique multilayered structure with a regular alternation of muscle and fibrous layer. Building on these findings, the current study aims to improve the scaffold properties by optimizing the decellularization process, thoroughly characterizing the ECM structure, and analyzing cellular behaviour during recellularization.

Methods

The bovine diaphragm scaffold, obtained by punch, was decellularized using various detergent protocols designed to preserve the ECM while clearing muscle fibers and maintaining scaffold structure. The effectiveness of decellularization was assessed through multiple assays, including residual DNA quantification, analysis of ECM protein content, evaluation of glycosaminoglycan fraction and quantification of the residual concentration. Histological analyses were also conducted to confirm fiber clearance and the preservation of the three-dimensional structure.

Results

The optimized detergent-enzymatic protocol successfully produced a muscle scaffold that preserves the structural integrity of the ECM while ensuring that the muscle fibers were effectively cleared to facilitate new cell seeding. DNA and muscle-specific proteins were effectively eliminated, while of ECM-specific proteins were preserved. Histological analysis further confirmed the retention of the scaffold's three-dimensional architecture, and chemical tests indicated the absence of detergent residues at potentially cytotoxic levels.

Conclusions

This work developed a refined and reproducible model that supports efficient cell integration and long-term viability, ultimately improving the applicability of bovine muscle organoids for in vitro muscle

organoids for in vitro muscle studies.

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_1023 In vivo application of a therapeutic ultrasound system for drug delivery applications in companion animals with spontaneous tumours. Kyriakos Spanoudes[1 2 3], Anastasia Antoniou[2 4], Antria Filippou[2 4], Nikolas Evripidou[4], Christakis Damianou[4]

[1]Department of Veterinary Medicine, University of Nicosia School of Veterinary Medicine, Nicosia, Cyprus; *[2]VET EX MACHINA LTD, Nicosia, Cyprus*; *[3]Cuprum Biotech, Larnaca, Cyprus*; *[4]Cyprus University of Technology, Limassol, Cyprus*

Introduction/Objectives

The objective of the study is to commercialise a Herein, Focused Ultrasound (FUS) ablation was utilised for enhancing drug delivery to spontaneous tumors in dogs and cats, employing a custom-made Magnetic Resonance-compatible FUS robotic system.

Methods

The system utilizes a single-element, spherically focused transducer operating at 2.6 MHz to non-invasively deliver high intensity ultrasonic energy to the tumor site. The efficacy of this technology was assessed through a trial involving 12 dogs and cats. All treatment procedures were performed successfully prior to tumor excision by the referring veterinarian.

Results

Histological examination of excised tumors revealed evidence of necrosis on the H&E-stained slides, visualized as well-defined regions of destroyed cell architecture. Overall, the FUS system demonstrated precise targeting capabilities, enabling partial ablation of tumors at various anatomical locations in veterinary patients. Notably, the system's portability facilitated its seamless integration into veterinary clinics. All participating animals remain under routine follow-up consultations.

Conclusions

The study outcomes revealed promising therapeutic potential, suggesting that the developed technology could provide a viable avenue for non-invasive therapeutic interventions and drug delivery in veterinary oncology for companion animals.

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_1291 Spontaneous repair versus Amniotic Epithelial Cells-Induced tendon regeneration in an Ovine Achilles Tendon Model: can Neural markers predict tendon healing outcome?. Valeria Giovanna Festinese[1], Melisa Faydaver[2], Delia Nardinocchi[2], Oriana Di Giacinto[2], Mohammad El Khatib[2], Annunziata Mauro[2], Maura Turriani[2], Paolo Berardinelli[2], Valentina Russo[2], Barbara Barboni[2]

[1]University of Teramo, University of Camerino, Camerino, Italy; *[2]University of Teramo, Teramo, Italy*

Introduction/Objectives

Recent evidence highlights the nervous system's role in tendon healing through neural markers Nerve Growth Factor (NGF), Neurofilament 200 (NF-200, marker of nerve ingrowth), Neuropeptide Y (NPY), Calcitonin Gene-related peptide (CGRP) and Galanin (GAL), which influence inflammation, cell proliferation and extracellular matrix (ECM) remodeling [1]. This study explored the predictive role of these neural markers in tendon healing using a validated ovine Achilles tendon injury model[2,3]. The

spatiotemporal expression pattern of these markers was compared in tendons undergoing amniotic epithelial stem cells-induced regeneration (AECs) vs. spontaneously healing tendons, 14 and 28 days post-injury (p.i.) [2,3].

Methods

An ovine Achilles tendon injury model was used under two conditions: AECs treatment and spontaneous healing (CTR). Neural markers (NGF, NF-200, NPY, CGRP, GAL) were analyzed for spatiotemporal expression patterns. Histological assessment of tendon healing and ECM remodeling analyzed tendon morphometric parameters (cell alignment, angle deviation) and collagen maturation, notably the ratio of Collagen type 1 (COL1) to Collagen type 3 (COL3) mRNA expression. Statistical analyses were conducted to assess relationships between neural marker expression and tissue organization.

Results

AECs-treated tendons showed enhanced ECM remodeling, with lower angle deviation at 28 days p.i. (AECs vs. CTR $p<0.05$), and better COL1/COL3 ratio at 14 and 28 days p.i. (AECs vs. CTR $p<0.01$). Spatiotemporal modulation of neural markers was observed in treated tendons compared to CTR. CTR tendons showed significant nerve ingrowth at 14 days ($p<0.05$); in treated groups, NF-200 expression remained lower ($p<0.05$), suggesting AECs regulate nerve ingrowth during tendon regeneration. NGF expression increased over time in CTR (14 vs. 28 days $p<0.05$) but stayed constant in AECs-treated tendons ($p<0.05$ vs. CTR 14 days).

Unlike CTR, neuropeptides expression was modulated in AECs-treated groups. NPY significantly increased in treated tendons from 14 to 28 days ($p<0.01$). CGRP and GAL significantly decreased in treated groups over time ($p<0.0001$); at 14 days their expression was higher than CTR ($p<0.05$).

Pearson's correlation analysis revealed strong positive correlations between NGF, CGRP and GAL expression. In AECs-treated groups lower levels of these markers positively correlated with better organization, unlike CTR, where higher neural markers levels were linked to delayed, disorganized healing.

Conclusions

The study highlights the predictive value of neural markers NGF, CGRP, and GAL in tendon healing, emphasizing their role as indicators of effective regeneration. AECs treatment showed a neuro-mediated mechanism, improving ECM remodeling, collagen maturation, and overall tissue organization. These results offer novel neurobiological insights into tendon regeneration, supporting the potential of biomarker-driven strategies to enhance therapeutic outcomes for tendinopathies.

1 Ackermann et al. Neuronal Pathways in Tendon Healing and Tendinopathy–Update. Frontiers in Bioscience Landmark 19:1251–1278 2014

2 Barboni et al. Achilles Tendon Regeneration Can Be Improved by Amniotic Epithelial Cell Allotransplantation, Cell Transplant 21:2377–2395 2012

3 Russo et al. Tendon Healing Response Is Dependent on Epithelial-Mesenchymal-Tendon Transition State of Amniotic Epithelial Stem Cells. Biomedicines 2022 19;10(5):1177

Topic: Veterinary medicine in TERMIS

Subtopic: Veterinary medicine in TERMIS

Type: Poster presentation

TERMIS25_1333 The granulation (t)issue in equine distal limbs culture of skin derived and exuberant granulation tissue (EGT) myofibroblasts to study cytoskeleton and extracellular matrix.

Jule Kristin Michler[1], Lena Partusch[2], Nadia Anantama[1], Ward Despiegelaere[2]

[1]Institute of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany; *[2]Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

Introduction/Objectives

In horses, wounds often have to heal by second intention wound healing. During the wound healing phases, a tissue bed of granulation tissue is built as in every wound. Especially in the lower limb (below carpus or tarsus), a fibroblastic wound healing disorder can occur: exuberant granulation tissue (EGT, also known as proud flesh or hypergranulation). These wounds are stuck between the inflammation and proliferation phase of the wound healing cascade, initially develop an epithelial rim but fail to close due to the rapid proliferation of highly vascularized, non-innervated tissue masses. Objectives of the work presented here are the thorough description of the extracellular matrix (ECM) in these wounds as well as the culture of EGT-derived myofibroblasts to elucidate their similarities and differences in 2D cultures compared to isolated fibroblasts from the skin of distal limbs.

Methods

Archived skin samples of the distal limb were used as positive controls. Cells obtained from these skin samples were in stock from a previous study. For setting up cell cultures from EGT, surgical waste material from five horses suffering of EGT wounds and in need of trimming the excessive tissue was harvested (Equine Clinic at Gent University and Leipzig University). For histopathology of the obtained samples, besides classical histological stains such as Hematoxylin & Eosin and Masson Trichrome, Herovici and Picrosirius red (PSR) stains were performed, the latter for evaluation using polarization microscopy. The tissue sections were then immunofluorescently stained using antibodies against Collagen I and III. To elucidate these markers in cultured cells, triple and quadruple stains of the named antibodies combined with cytoskeletal proteins were performed: anti-alpha smooth muscle actin (α-sma) for actin filaments, anti-Vimentin for intermediate filaments (class III) and anti-ß-tubulin for microtubules.

Results

The ECM was initially described histopathologically. A clear distinction of the superficial (inflammatory) and deep (fibrotic) layer of the EGT sections was seen in the histological assessment and backed up by the PSR and anti-Collagen I immunofluorescent stains. Although described in literature, the anti-Collagen III-antibody did not lead to reliable results.

From the EGT samples, three cultures were successfully isolated. Two approaches were lost due to the heavily infected tissue and no washing steps or triple antibiosis secured the cultures.

The growth characteristics (population doubling time (PDT) did not differ in 2D cultured skin- and EGT-derived cells.

The majority of cells expressed α-sma, Vimentin and Collagen I with a semiquantitatively increased expression of α-sma and Collagen I for the distal limb fibroblasts, whereas Vimentin was expressed in comparable levels.

Conclusions

Cultures of activated cutaneous (myo)fibroblast feature the characteristics of fully differentiated wound healing myofibroblasts. Further aims should include the development of a 3D culture approach to better mirror the in vivo situation and the coculture with immune cells as well as MSC to study their potential beneficial effect in wound healing. Basic research in this field and appropriate models to study treatment options are needed to lower the fatal 25% of horses with EGT wounds that cannot be healed.

Published online: June 12, 2025