A FIBRINOGEN-BASED BIOINK FOR SKIN TISSUE ENGINEERING

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Background

The 3D bioprinting enables to prepare skin tissue engineered grafts for non-healing chronic wounds or skin defects leading to effect on patients quality life[1]. Fibrin structural composition and binding to cells highly determine wound healing process[2].

Objective

The aim is to develop a fibrinogen-based bioink with addition of alginate for skin constructs printing followed by fibrin cross-linking using a thrombin–calcium chloride ($CaCl_2$) solution.

Methods

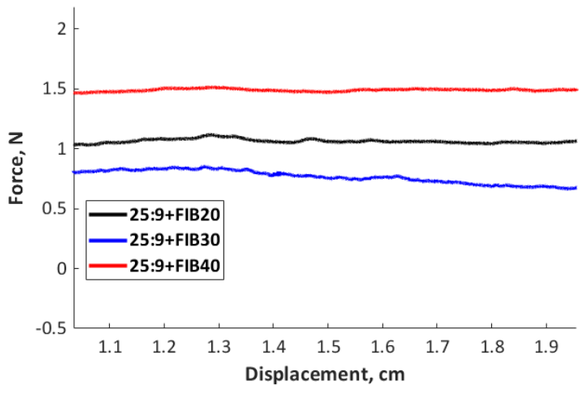
Several concentrations of bovine fibrinogen (FIB-20-30-40 mg/ml) and sodium alginate were dissolved in dH_2O and semi-crosslinked mixing with 100mM $CaCl_2$ solution at volumetric ratio of 25:9. The crosslinking-solution consists in bovine thrombin (50UT/ml) dissolved in 50mM $CaCl_2$. The physical and biological properties of bioinks were characterized.
The bioink that showed the best parameters was mixed with human dermal fibroblasts ($6x10^6$cells/ml) and human epidermal keratinocytes ($8x10^6$cells/ml) to print a bi-layered fibrin skin constructs. The bio-printed constructs were cultured up to 14 days.

Results

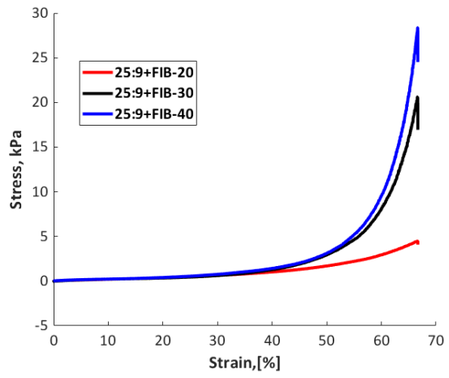
Bioink characterization

The fibrinogen based bioinks resulted homogeneous (i.e. constant force in homogeneity test) with compressive modulus (mechanical test) that increases according to the fibrinogen concentration augment as well as the pores dimensions (SEM Images).

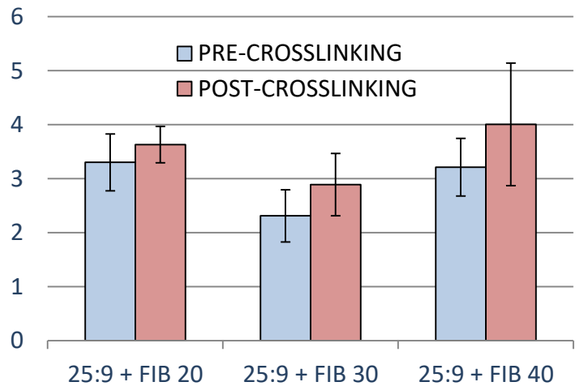
HOMOGENEITY TEST



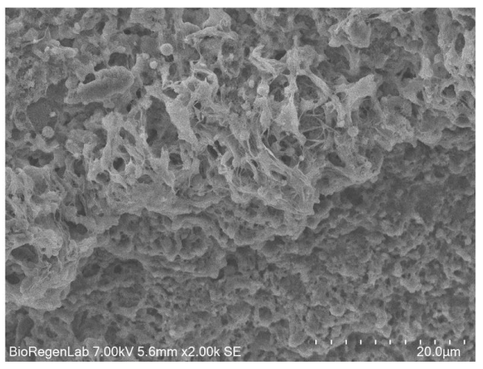
COMPRESSIVE MECHANICAL TEST

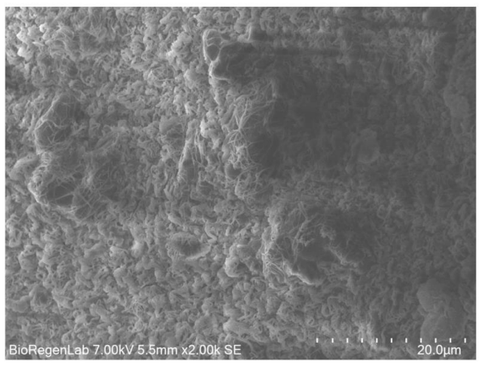


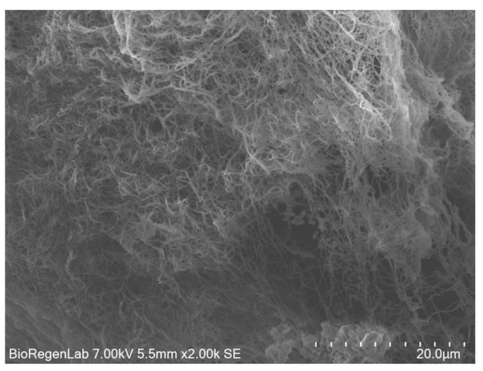
SPREADING RATIO



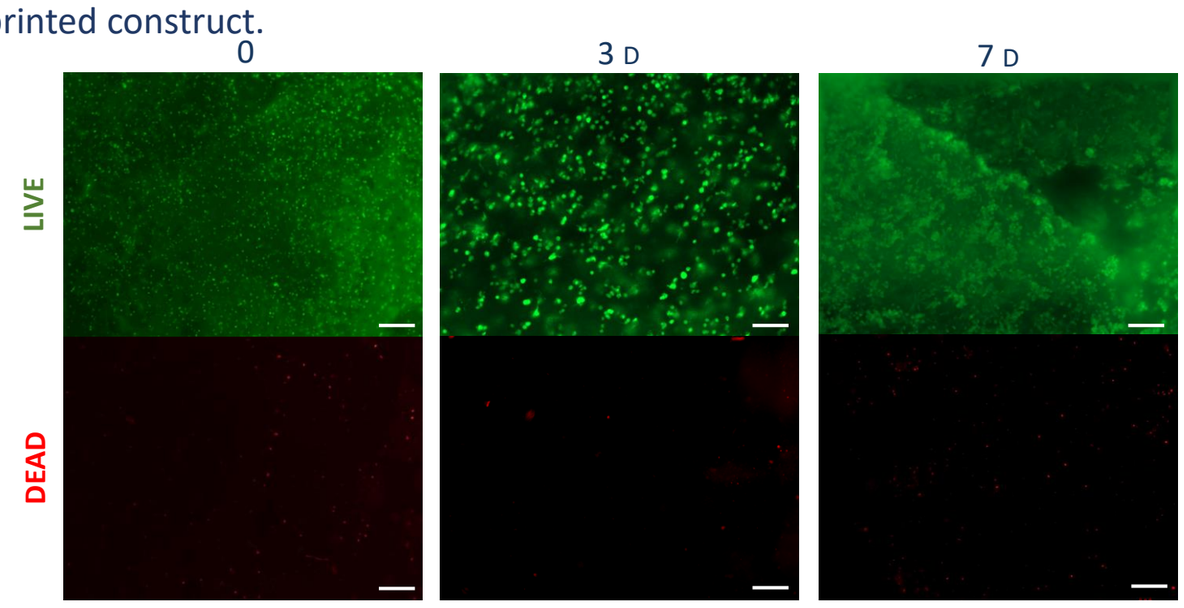
MORPHOLOGY-SEM IMAGES





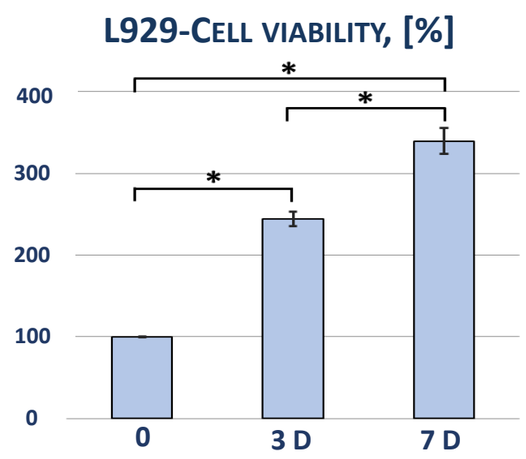


The 25:9+FB30 was the bioink with the lowest spreading ratio selected for biocompatibility evaluation with L929 mouse fibroblast ($4*10^6$cell/ml) showing good cell proliferation (LIVE/DEAD and cell viability assays) within the 3D printed construct.



Scale bar 100µm

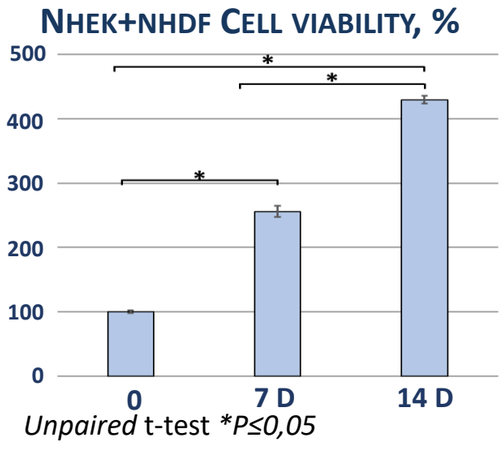
L929-CELL VIABILITY, [%]



Unpaired t-test **P≤0,05*

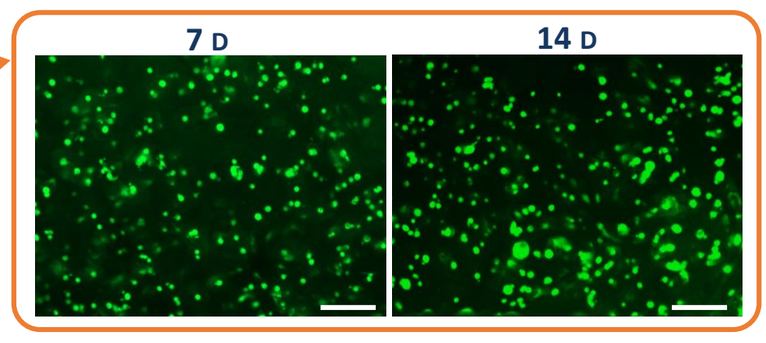
Bi-layered fibrin skin construct characterization

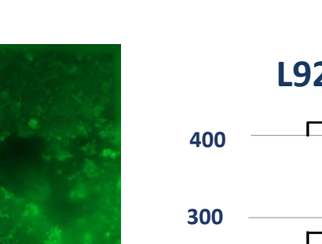
NHEK+NHDF CELL VIABILITY, %



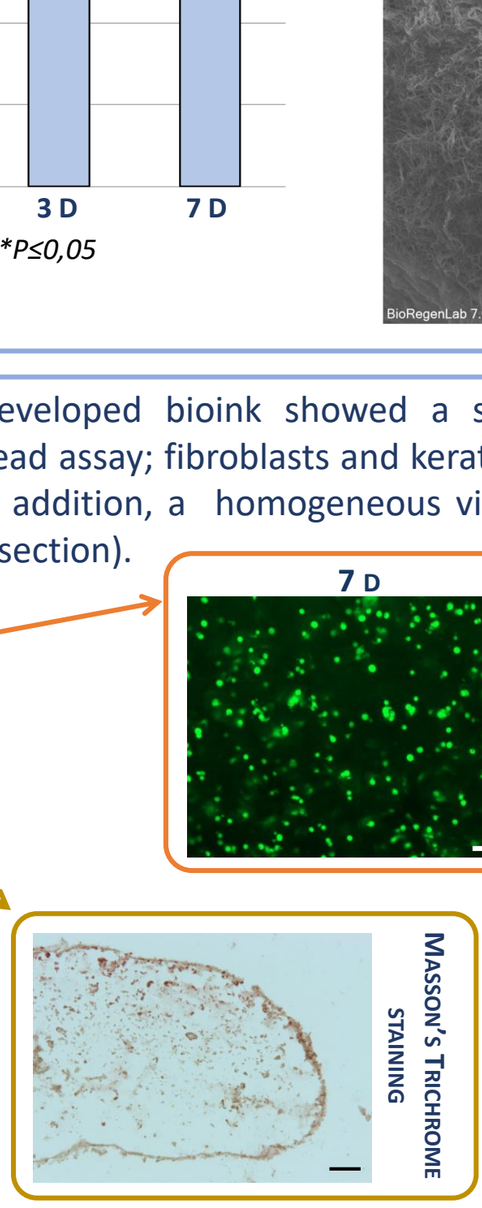
Unpaired t-test **P≤0,05*

The bio-printed constructs using the developed bioink showed a statistically significant cell proliferation rate confirmed also by live/dead assay; fibroblasts and keratinocytes clusters diameter increased at day 14 respect to day 7. In addition, a homogeneous viable cells distribution was observed in sample thickness (histological section).





Scale bar 100µm



Conclusion

These results showed that the fibrinogen-based bioink (25:9 as ratio between sodium alginate and $CaCl_2$ with 30 mg/ml of fibrinogen) has good printability properties enabling human cell proliferation and tissue maturation. The bi-layered fibrin skin construct could be used as skin graft in wound healing applications.

Acknowledgements

This study was supported through Dr. Vassili Fotis private donation.

References

[1] Falanga V. Lancet, (2005).
[2] Hoppenbrouwers T, et al. Thrombosis research, (2017)