Cell-laden *Prionace glauca* Skin Collagen-based 3D Printed Scaffolds for the Engineering of Mineralized Tissues

Gabriela S. Diogo[1,2], Catarina C. Marques[1,2], Carmen G. Sotelo[3], Ricardo I. Pérez-Martín[3], Rogério P. Pirraco[1,2], Rui L. Reis[1,2,4] and Tiago H. Silva[1,2]

[1] 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra 4805-017 Barco, Guimarães, Portugal; [2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3] Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain; [4] The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal.

INTRODUCTION

Composite scaffolds, namely collagen-apatite based materials, have been widely employed in the biomedical field envisioning bone tissue regeneration, commonly based on the direct mixing of calcium phosphates powder and collagen solutions or scaffolds mineralization by immersion in simulated body fluid (SBF). However, direct mixing is an uncontrolled method and can result in a lack of homogeneity of particle distribution, while SBF method normally requires long times of incubation and mineralization is mainly limited to surface, which might result in a limited bioactivity. Alternatively, we propose for the first time the *in situ* mineralization of *Prionace glauca* skin collagen (PGColl) and its use on inks for the 3D printing of encapsulated cells envisioning mineralized tissue applications. To induce hydroxyapatite formation through PGColl fibrils, calcium chloride and ammonium hydrogenphosphate were added to solubilized collagen [1]. Mineralized collagen was combined with alginate and cells to obtain novel bioinks used on the production of 3D structures by using a Regemat3D bioprinter.

MATERIALS AND METHODS

Biomimetic mineralization of shark collagen

Regemat3D bioprinter for cell-laden hydrogels fabrication

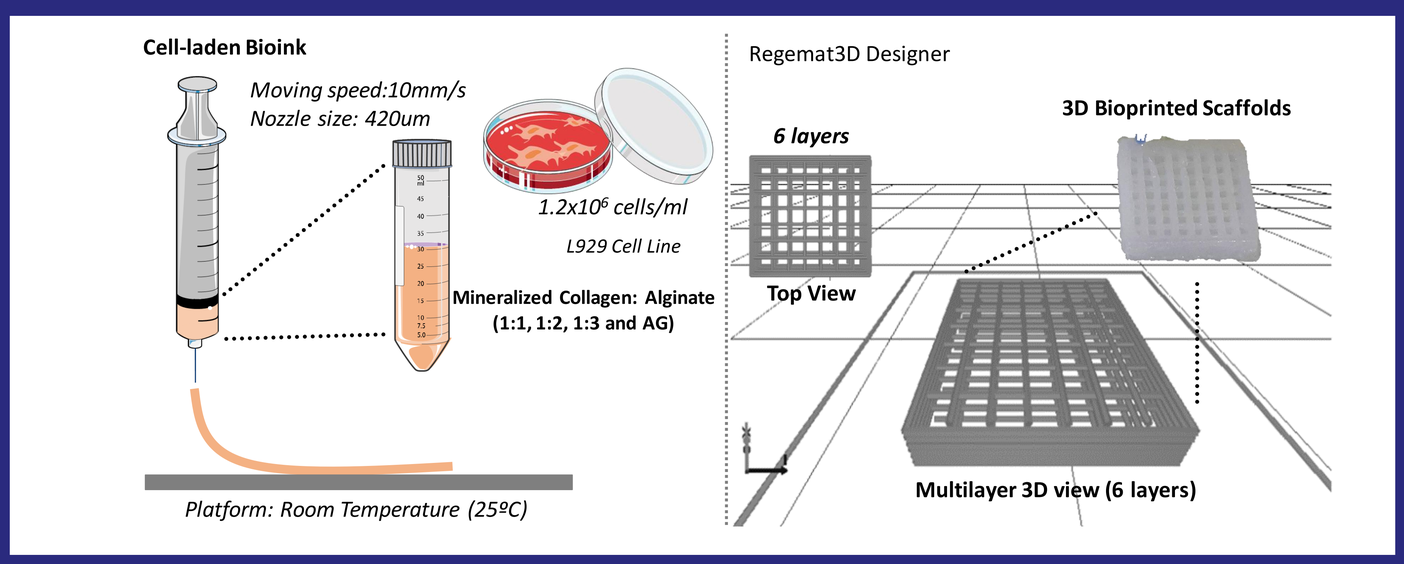

Figure 1. Schematic representation of the process to fabricate cell-laden mineralized collagen:alginate hydrogels by using a Regemat3D printer dispensing system.

Characterization

- ✓ Confirmation of collagen mineralization by:
  - X-ray diffraction (XRD)– To investigate the crystallinity;
  - Fourier Transform-Infrared (FT-IR) Spectroscopy (FTIR) – to determine the chemical composition;
- ✓ Thermogravimetric analysis (TGA) - to determine the calcium content;
- ✓ Rheological properties - oscillatory and viscosity assays;
- ✓ Biological Performance:
  - Live/dead assay
  - Metabolic activity (MTS)
  - DNA Quantification

RESULTS AND DISCUSSION

Characterization of Mineralized Collagen, Inks and Hydrogels

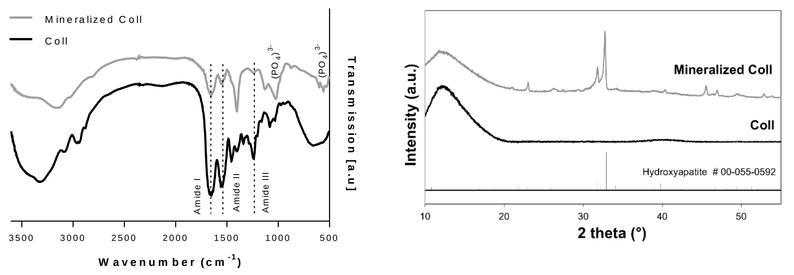

Figure 2. A) FT-IR spectra and B) XRD of pure (black) and mineralized (grey) PG collagen. The presence of phosphate groups confirmed the efficiency of the mineralization process.

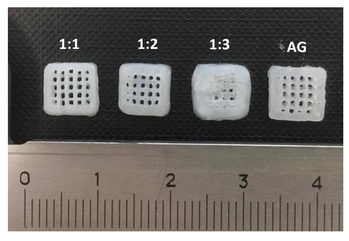

Figure 5. Photograph of the printed hydrogels: from left to right, decreased concentrations of mineralized collagen

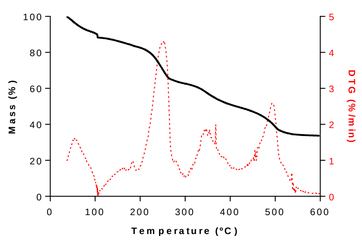

Figure 3. Characteristic oxygen DTG curve. Hydroxyapatite corresponding to 33,43 % ± 0,70 of the total sample.

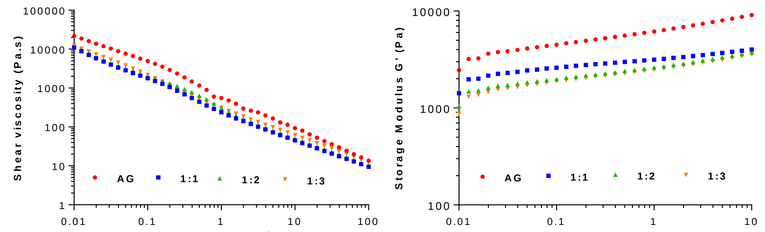

Figure 4. Influence of mineralized PG collagen: alginate concentration on rheological properties measured at 37ºC. At left it is represented the different bioinks viscosity and at right the storage Modulus (G').

Cell Response in Cell-Laden Hydrogels

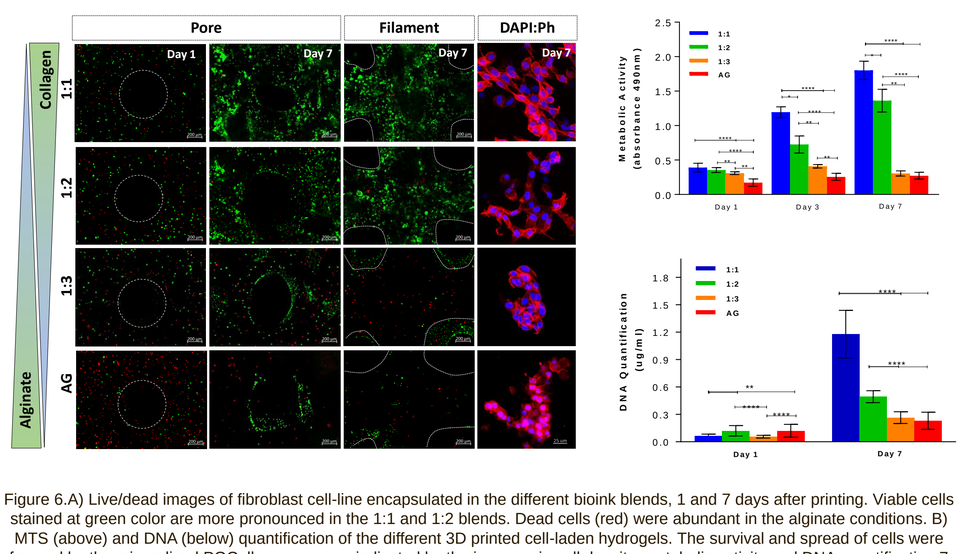

Figure 6.A) Live/dead images of fibroblast cell-line encapsulated in the different bioink blends, 1 and 7 days after printing. Viable cells stained at green color are more pronounced in the 1:1 and 1:2 blends. Dead cells (red) were abundant in the alginate conditions. B) MTS (above) and DNA (below) quantification of the different 3D printed cell-laden hydrogels. The survival and spread of cells were favored by the mineralized PGColl presence, as indicated by the increase in cell density, metabolic activity and DNA quantification 7 days after cell culture.

CONCLUSIONS

The *in situ* mineralization of PGColl by using a co-precipitation method was successfully achieved. The printed cells in the different bioink blends were homogeneously distributed through all constructs, which indicates a successful mixing of the cells in the different ink blends. Nevertheless, significant differences in terms of cell viability were detected between the collagen-based bioinks when compared with alginate alone. The intensity of the red signal decreases as alginate ratio decreases, followed by enhanced cell metabolic activity. Such result indicates that a mechanically stable and cell-viable construct requires a bioink comprising a limited range of rheological properties. High blends viscosity requires high printing pressures to allow extrusion, which induces cell-damaged due to the high shear stress. The PGColl-based constructs shown to support printing and proliferation of fibroblast cell line. The survival and spread of cells were favored by the presence of mineralized PGColl.

References:

[1] Hoyer, B., et al., Biomimetically mineralized salmon collagen scaffolds for application in bone tissue engineering. Biomacromolecules, 2012. 13(4): p. 1059-1066.

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