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Cell-laden Biomimetically Mineralized Shark Skin Collagen-based 3D Printed Scaffolds for the Engineering of Hard Tissues

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INTRODUCTION: 3D approaches made of collagen-apatite materials have been targeted envisioning mineralized tissues applications. Direct mixing of calcium phosphates powder with collagen solutions or scaffolds immersion in simulated body fluid (SBF) are the most common methods. However, loss of efficiency has been reported, pointing to uncontrollable methods and long processing time, which might result in a limited bioactivity. Alternatively, we propose for the first time the *in situ* mineralization of *Prionace glauca* (blue shark) skin collagen (PGColl) and its use on inks for the 3D bioprinting of encapsulated cells envisioning the engineering of mineralized tissues.

METHODS: Collagen extracted from blue shark skin through an acidic method was solubilized in 10 mM HCL. To induce hydroxyapatite formation, calcium chloride and ammonium hydrogenphosphate were added as calcium and phosphate sources (Calcium-to-phosphate ratio =1.67), respectively. To prepare the cell-laden bioinks, 2% (w/v) PGColl was combined with 12% (w/v) alginate at the following volume ratios: 1:1, 1:2, 1:3 and only alginate (AG). The printing process was carried out using sterile material in a REGEMAT 3D dispensing system, at room temperature (25 ºC).

RESULTS: FT-IR and XRD analyses confirmed hydroxyapatite formation of the *in situ* mineralized collagen. The printed constructs using the different bioinks exhibited a homogeneously distribution of cells, which indicates a successful cell encapsulation. The intensity of the red signal (dead cells) decreased with alginate ratio, revealing the positive effect of mineralized PGColl with enhanced cell metabolic activity and proliferation (DNA quantification).

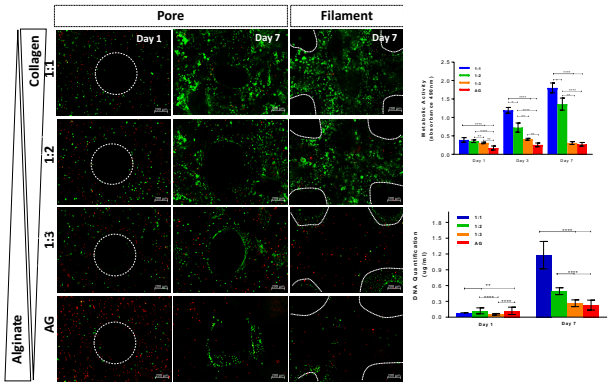

Figure 1. Live (green) / Dead (red) assay performed 1 and 7 days after cell culture, i.e., bioprinting (left); MTS assay and DNA quantification (right up and down, respectively).

DISCUSSION & CONCLUSIONS: Successfully designed and printed cell-laden constructs made of *in situ* mineralized PGColl through a biomimetic approach were achieved. The survival and spread of cells were favored by the presence of mineralized PGColl.

REFERENCES: Xia Z. *el tal,* J. Mater. Chem. B, 2014. 2(14): p. 1998-2007.