Bulk Biomineralization of Dense Fibrillar Collagen-Bioglass® Hybrid Scaffolds

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Introduction: Successful scaffolds for bone tissue engineering (BTE) should satisfy requirements such as biocompatibility, osteoconductivity, osteoinductivity, biodegradability, and appropriate biomechanical properties. Hybrid materials, such as a combination of type I collagen and 45S5 Bioglass® (BG) may meet these requirements. Type I collagen is the main organic component of bone, and its biocompatibility, biodegradability and biological properties are well documented, especially in its fibrillar gel form. However, collagen fibrillar gels present low mechanical properties and low collagen fibrillar density, relative to the native tissues. To overcome this, Brown *et al.* [1] developed a processing technique based on unconfined plastic compression (PC), to rapidly fabricate dense collagen matrices, which mimic the extracellular matrix microstructural, and biological properties as well enhancing the mechanical properties via the controlled increase in nano-fibrillar density. Moreover, suitability of PC for BTE purposes was previously assessed [2], and cellular PC collagen gels were tested *in vivo* [3]. BG is osteoinductive and osteoconductive and it bonds to both soft and hard tissues. In this work, rapidly fabricated dense nano-fibrillar collagen-Bioglass® hybrid gels were produced to investigate their potentiality for BTE purposes, as rapidly implantable scaffolds. Their rapid processing eliminates the long-term culture required for cellular induced remodeling and mineralization of scaffolds. This study assessed the potential for BG to induce three-dimensional mineralization of dense collagen (DC) gel, *in vitro,* by conditioning in simulated body fluids (SBF).

Methods: The hybrid gels were prepared incorporating BG particles (d≅5µm) in the collagen solution prior to gelling (30minutes, 37°C). Two BG contents were used: 40 and 60 dry weight% (DC-B 60-40 and DC-B 40-60), while DC gel was used as control. Gels underwent to PC (1kPa, 5minutes) and then were conditioned in SBF at 37°C for up to 14 days. SBF was replaced every 2 days. Morphological characterization of the different gels was achieved with micro computed tomography (µCT) (SkyScan 1172) and scanning electron microscopy (SEM) (FEG-SEM 4700, Hitachi) analyses. The chemical structure of hybrid gels and control was investigated with attenuated total reflectance Fourier transform infrared (ATR-FTIR) microscopy (Spotlight 400, Perkin Elmer) and x-ray diffraction (XRD) (X8 advance, Bruker) both on the surface and in the cross section of the samples. Moreover, ATRFTIR microscopy was used to semi-quantify the biomineralization extent achieved at different time in SBF and BG concentrations. Tensile tests (ElectroForce® Biodynamic® Test Instrument 5160, Bose Corp.) were conducted to investigate changes in the mechanical properties with time in SBF and BG concentration. All the results were statistically compared with two way ANOVA tests.

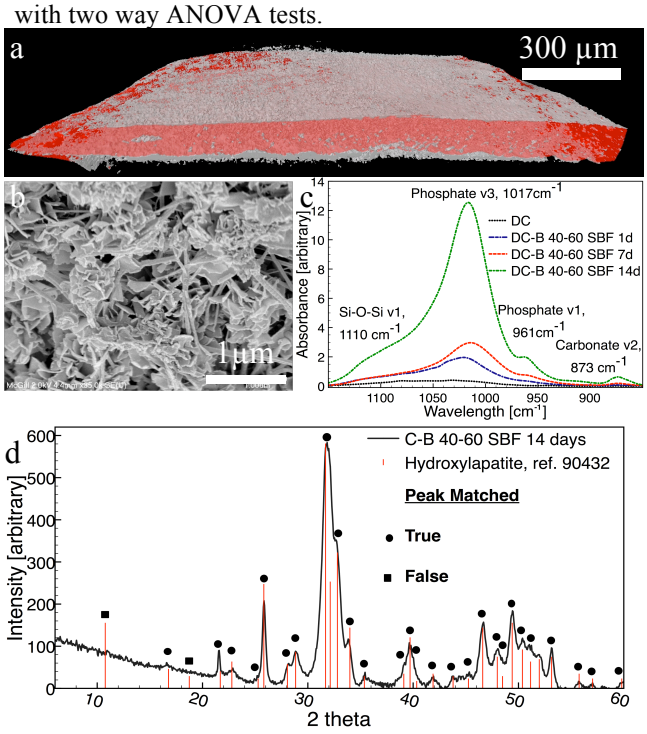

Figure1: *Morpho-chemical analysis of DC-B 40-60 samples after 14 days in SBF. a)µCT 3D reconstruction The HCA phase is highlighted in orange; b)SEM micrograph showing collagen nanofibres and HCA crystals; c)ATR-FTIR spectra showing the increase of v3PO_4^{3-} and v2CO^{3-} peaks, typical of HCA, with immersion time in SBF; d) XRD executed on the cross-section confirming the presence of HCA.*

Results and Discussion: Bulk nucleation and growth of calcium-phosphate crystals were visible from µCT, SEM (Figure 1a and b) and ATR-FTIR microscopy. Mineralization was detected at day 1 in SBF, while ATR-FTIR microscopy and XRD (Figure 1c and d) revealed the presence of hydroxyl-carbonated apatite (HCA) on the surface and within the two hybrid scaffolds at days 7 and 14. Principal component analysis executed on ATR-FTIR microscopy revealed that the mineralization extent was a function of both BG concentration and immersion time in SBF. Mineralization of DC gels occurred in a lower extent. ATR-FTIR and SEM confirmed the triple helical structure and typical banding pattern of fibrillar collagen. Tensile mechanical analysis showed an increase in stiffness as imparted by mineralization of scaffolds as a function of time in SBF and BG concentration.

Conclusion: These results demonstrated the achievement of a hybrid-mineralized matrix that is potentially suitable for bone tissue engineering.

References: 1.Brown, R.A. Adv. Func, Mat., 2005; 15(11):1762-1770; 2. Buxton, P.G. Bone, 2008; 43(2):377-385; 3. Mudera, V. J. Tissue Eng. Regen. Med. 2007; 1:192–198

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