Dense Collagen-Nanosized Bioactive Glass Hybrid as Rapid Implantable Scaffold for Bone Tissue Engineering

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Statement of Purpose: Plastic compression (PC) is a processing technique geared to rapidly fabricate (t=30 min) dense collagen (DC) gels, which mimic the extracellular matrix (ECM) microstructural, and biological properties, as well as enhancing the mechanical properties via the controlled increase in collagen fibrillar density [1]. DC gels may either be used as an *in vitro* osteoid-model to investigate collagen mineralization or as a scaffold for bone tissue engineering (BTE). We have recently demonstrated that the incorporation of micro-sized Bioglass® 45S5 (μBG) in DC gel led to the formation of a hybrid matrix that is potentially suitable for BTE, due to its bulk mineralization properties, when exposed to simulated body fluid (SBF) [2]. In this scenario, the use of nano-sized bioactive glass (nBG) represents a substantial improvement, due to the higher reactivity of this glass, corresponding to an accelerated mineralization [3]. Moreover, the size of nBG may result its tighter interaction with the DC nanofibrils and in a more homogenously distributed apatite nucleation within the collagen fibrils. Furthermore, nBG may induce an *ab initio* stiffer gel even at the nanoscale (*i.e.* the scale at which the cell feels) and a more homogenous ion release within the gel, which may influence cell fate.
Therefore, this study assessed the potential of nBG in inducing rapid three dimensional (3D) mineralization of DC gel by conditioning in SBF, and the effect of this mineralized matrix on the fate of a pre-osteoblastic cell line cultured without osteogenic factors.

Methods: The hybrid gels (DC-nBG) were prepared by incorporating nBG particles (same composition of 45S5 Bioglass® [3], d≅5-20nm, 40 dry weight %) in the collagen solution prior to gelling, as previously described [2]. DC was used as control. Rectangular gels underwent PC and were rolled to form a rod like structure (∅=1.5mm, l=8mm) (Fig. 1A). Acellular biomineralization was investigated by conditioning the scaffolds in SBF up to 7 days. Morphological characterization of the hybrid was achieved with micro computed tomography (μCT) and scanning electron microscopy (SEM). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) microscopy and x-ray diffraction (XRD) were used to investigate the chemical structure of DC-nBG gels as a function of time in SBF. The compressive modulus of the gels was evaluated as function of time. Moreover, the effect of nBG in a cellularized osteoid model was investigated up to 21 days using MC3T3 pre-osteoblast cultured in the absence of osteogenic media. Seeded cell morphology in the hybrid gel was studied with SEM and laser scanning confocal microscopy (LSCM) analyses, while cell viability and metabolic activity were investigated with Calcein AM-Ethidium homodimer-1 and AlamarBlue™ (AB) assay, respectively. MC3T3 induced gel contraction was monitored with μCT volumetric analysis using the same wet samples (n=3) up to day 21. Cellular alkaline phosphatase production was evaluated as a indication of osteoblastic differentiation.

Results: Mineralization was detected after 1 hour in SBF, while carbonated-hydroxylapatite (CHA) was formed on the surface and within the hybrid scaffold at day 1. Bulk nucleation and growth of calcium-phosphate crystals were visible from μCT, SEM and ATR-FTIR microscopy (Fig. 1B). This result confirms the high reactivity of the nBG, when compared to μBG where CHA was obtained after 7 days in SBF [3].

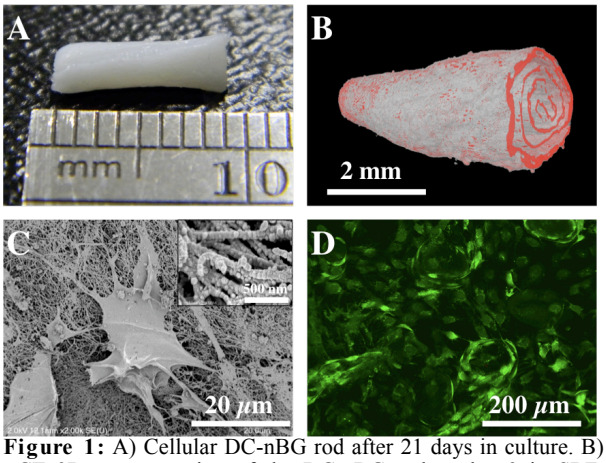

Figure 1: A) Cellular DC-nBG rod after 21 days in culture. B) μCT 3D reconstruction of the DC-nBG rod at day 3 in SBF. CHA rich areas are highlighted in red. C) SEM micrograph of MC3T3 at day 21 in DC-nBG rod. Inset image showing matrix mineralization. D) Maximum intensity projection of Calcein AM and EtBR-1 binding LSCM micrograph showing living MC3T3 in DC-nBG rod at day 21.

Furthermore, mineralization occurred homogeneously in the hybrid rod (Fig. 1C). The incorporation of nBG and the consequent rapid mineralization of the matrix was also reflected in the increased compressive modulus. In addition, MC3T3s were viable across the thickness of the rod up to at least day 21, as seen from maximum intensity projection of Calcein AM florescence and EtBR-1 binding (Fig. 1D). AB assay suggested a difference in cell activity cultured in the presence of nBG, when compared to DC gel, due to a significantly lower AB reduction in DC-nBG gels ($p<0.05$). This result along with the different cell morphology reported at day 7, as seen from LSCM and SEM, suggests a different fate of MC3T3s cultured in the presence of nBG, probably due to the rapidly mineralized matrix and the nBG degradation related ion release. μCT investigation of scaffold volumetric contraction revealed a significant decrease in cell-mediated contraction of DC-nBG gels when compared to the control.

Conclusions: Rapidly produced dense collagen-nanosized bioactive glass hybrid gels are potentially suitable as scaffolds for immediate implantation for BTE. Their biomineralization takes place within hours when exposed to SBF and culture medium. Furthermore, the presence of nBG in DC gels may modulate cell fate, which requires further analysis.

References: 1. Brown, R.A et al., Adv. Func, Mat., 2005, 15 (11), 1762-1770; 2. Marelli et al., Biomacromolecules, 2010, 11 (6), 1470-1479; 3.Misra et al., Biomaterials, 2010, 31 (10), 2806-2815

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