Material Properties and Osteogenic Potential of CaP Mineralized Collagen-Glycosaminoglycan Scaffolds

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Statement of Purpose: This work describes the development of mineralized collagen-GAG (CGCaP) scaffold for regeneration of critically-sized bone defects. Our approach emphasizes rigorous material characterization coupled with a long-term *in vitro* culture to better understand the material-cell interaction. We hypothesize that the inclusion of a mineral component will drastically change both material properties and induce osteogenic differentiation and new matrix synthesis for MSCs independent of traditional osteogenic media supplementation.

Methods: Non mineralized collagen-GAG (CG) scaffolds were fabricated via lyophilization from a suspension of collagen and chondroitin sulfate in acetic acid. Alternatively, collagen and GAG were mixed with phosphoric acid with calcium salts to produce mineralized CG (CGCaP) variants. We assessed scaffold composition, microstructure, mechanical behavior, and permeability for all variants. CGCaP composition was determined via hydroxyproline and DMMB assays for the quantification of collagen and GAG, with CaP content determined via a mass subtraction. CaP mineral phase was determined via powdered x-ray diffraction. Average pore size of all samples was determined via aniline blue stained histology specimens. Scaffold mechanical properties were determined via unconfined compression for both hydrated and non-hydrated scaffolds. Permeability of scaffolds was investigated via the constant head permeability test and Darcy's law [1]. Scaffold osteogenic potential was assessed via *in vitro* culture of human mesenchymal stem cells (hMSCs) over an 8 week period using different media treatments: growth media, BMP2 enriched (100 ng/mL) growth media, osteogenic media. Cell metabolic activity was measured using an AlamarBlue assay, while osteogenesis was assessed via PCR (BSP, OP, COL1, and RUNX2 expression). Changes in scaffold mechanical properties due to remodeling were monitored via unconfined compression, and changes in CaP mineral content were determined via histology (alizarin red) and microCT analysis. Statistics were performed using two-way ANOVA with significance set to $p < 0.05$.

Results: Compositional analysis confirmed the ability to create CGCaP with a range of mineral content (40, 80 wt% CaP) (Fig 1). All CGCaP scaffold variants contained the brushite phase of mineral. Interesting, we observed slow release of Ca and P ions into culture media for CGCaP scaffolds, with mineral release reaching a stable plateau over the course of 3 weeks. CG and CGCaP average pore sizes were found to be 162 um and 152 um respectively with no variation with lyophilization conditions (-10°C vs -40°C) (Fig 2). CG and CGCaP scaffolds behaved as low-density open-cell foams under mechanical load. Further, scaffold elastic moduli were found to be 8.8 +/- 1.1 kPa and 159.0 +/- 18.3 kPa for CG and CGCaP scaffolds, respectively (Fig 1). Finally, CG and CGCaP permeability was identified as 6.1E-7 and 2.4E-8 m^2 respectively. As expected, scaffold permeability decreased with applied strain (0 – 65%).

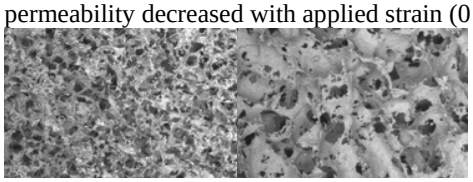

Figure 1. SEM of 40 (left) vs 80 (right) wt% CGCaP

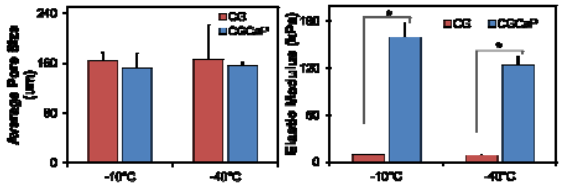

Figure 2. Pore size and non-hydrated elastic modulus

All scaffolds supported metabolic expansion of MSCs in culture over 8 weeks, though, though MSC metabolic activity was significantly reduced for osteogenic supplementation (Fig 3). Gene expression analyses showed up regulation of COL1A1 in CG and CGCaP scaffolds, but selective up regulation of BSP in CGCaP scaffolds, even for growth media. Increases in scaffold stiffness and mineral CaP content indicated significant remodeling and osteogenesis in CGCaP scaffolds (Fig 4).

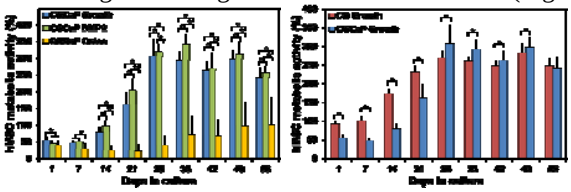

Figure 3. Metabolic activity

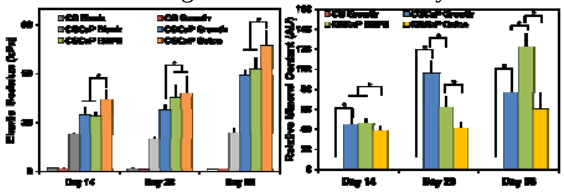

Figure 4. Changes in modulus and mineral content

Conclusions: We have demonstrated a mineralized CG (CGCaP) scaffold that can selectively induce MSC osteogenic differentiation without the need for conventional osteogenic supplementation. We have also described microstructural, mechanical, and permeability properties. Ongoing efforts are exploring CGCaP scaffold osteogenic potential for adipose-derived MSCs and incorporation of the CGCaP scaffold into PCL structures to balance mechanical and bioactivity concerns.

References: [1] Weisgerber et al., *J. Mech. Behav. Biomed. Matls.*, 28:26-36, 2013.