Uniform Three-Dimensional Biomineralization of Protein Incorporated Mineral Layer on Porous Polymer Scaffolds

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Statement of Purpose: Presence of biomimetic apatite on polymeric scaffolds increases the amount of bone regeneration *in vivo* compared to polymer controls following explantation of progenitor cells [1]. However, biomimetically precipitating apatite onto three-dimensional scaffolding has not been well established. Surface modification of the polymer template via hydrolysis or aminolysis can lead to faster mineralization, but still requires lengthy incubation times (2 weeks) and often will not achieve uniform mineralization throughout the thickness of a scaffold. Furthermore, spatial control of biomolecular incorporation has been achieved on biomimetically precipitated coatings in 2 dimensions [2]. Incorporating biomolecular signals into the biomimetic mineral layer in three dimensions (3D) could prove effective for faster and higher quantity bone growth. This study aimed to prove that flow induced mineralization of simulated body fluid (SBF) through porous scaffolds would promote the formation of a uniform mineral layer in 3D. This study also investigated whether the bioreactor could be used to co-precipitate a model protein (bovine serum albumin, BSA) into the 3D scaffolds.

Methods: 95% porous scaffolds (2x10mm diam.) were fabricated in a Teflon® or Delrin® mold via salt-leaching using 7.5w/v% 85:15 PLGA-chloroform solution and 450-600um diam. salt particles. The scaffolds were dried for 48h and leached in dH_2O for 36h. Scaffolds were treated with 0.5M NaOH for 7 min. and rinsed with ddH_2O. The filtration system designed is shown in Fig. 1.

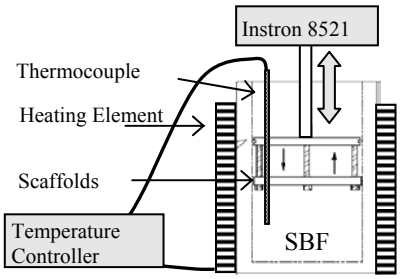


Figure 1: Schematic showing filtration device and components. Four scaffolds were mineralized in the system. (system capable of mineralizing 16)

SBF solutions (1x-SBF: 141mM NaCl, 4mMKCl, 0.5mM $MgSO_4$, 1mM $MgCl_2$, 4.2mM $NaHCO_3$, 2.5mM $CaCl_2 2H_2O$, 1mM KH_2PO_4: 2x and 5x SBF are multiples of 1x) were warmed to 37°C prior to each experiment. 1L of 5x-SBF (pH=6.4 at 25°C) was introduced for 12 hrs, being changed after 6 hrs. One liter of 2x-SBF (pH=6.8 at 25°C) replaced the 5x-SBF and was replenished every 12 hours. A fatigue program cycled the mold with scaffolds (n=4) at a 25.4mm amplitude at 0.0011Hz for 5 days in 37°C solution. Two control groups [submerged static (n=4) and floating static (n=4)] were mineralized and subjected to the same SBF regimen (0.5L SBF instead of 1L) in a 37°C incubator for 5 days.

After 5 days, all scaffolds were removed, rinsed in ddH_2O, and dried. Scaffolds were scanned (n=4) in a Micro-computed tomography (MicroCT) system (EVS MS8X-130, 16um voxel size) to obtain mineral volume % (MV%) (MicroView®) and 3D rendered images.

Co-precipitation of BSA was determined by incorporating a 1:5 ratio of FITC-BSA:BSA in the 2x-SBF, stopping the mineralization after 3 days. The initial concentration of BSA in solution was 200ug/mL. Amounts of FITC-BSA were read on a UV Spec. (n=4). The effect of filtration on MV% and BSA incorporation were determined using Mann-Whitney Rank Sum t-test.

Results: Filtration significantly increases mineral deposition compared to both submerged and floating control groups. While filtration increased the overall MV %, uniform mineralization throughout the thickness of the filtration scaffold was also achieved (Fig. 2).

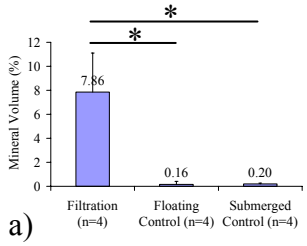
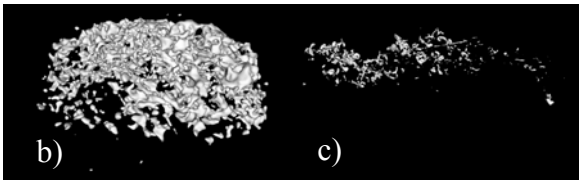


Figure 2: (a) MV% showing filtration mineralized the greatest. * - (p = 0.029) MicroCT 3D renderings [threshold value = 1000] of filtration (b) and submerged (c) showing a difference in mineral deposition.



Increased co-precipitation of BSA in 3D was also achieved (Fig. 3). Incoroporation of BSA in the filtration scaffolds was ~4x greater than submerged controls.

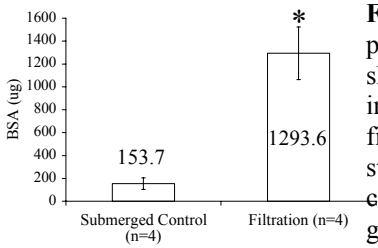


Figure 3: Co-precipitated BSA values showing greater incorporation in the filtration scaffolds. * - statistically significant compared to control groups ($p<0.001$)

Conclusions: Flow increased and induced uniform 3D mineralization. Uniformly coating a 3D polymer scaffold template with a hydroxyapatite layer could promote 3D osteogenesis *in vivo* through either cell explantation or conductive properties of the mineral. Furthermore, this system can be utilized in an osteoinductive strategy to incorporate biomolecules (proteins, peptides, growth factors, etc.) into the mineral layer that have the ability to influence the adhesion, proliferation, and differentiation of introduced cells towards an osteoblastic lineage.

References: [1] Kohn et al., Int. Conf. Chem. Biol. Min. Tissue, in press. [2] Luong et al. Biomat. 2005, Aug 30 online. This work is supported by NIH R01 DE 015411 and the Tissue Engineering at Michigan Grant DE07057.