Rapid Mineralization of Cell-Seeded Collagen-Hydroxyapatite Composite Scaffolds via a Biomimetic Process

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Statement of Purpose: Addition of hydroxyapatite (HA) and biomimetic mineralization has been used to increase the biochemical and mechanical function of injectable composite materials [1] and protein scaffolds [2]. However, the mineralization process is typically performed in the absence of cells, since the conditions used can be cytotoxic [3]. Here we present results of a study in which cell-seeded protein scaffolds were mineralized by both incorporating HA and promoting exogenous mineralization using simulated body fluid (SBF). The goal of this work was to enhance the mechanical stability of collagen-HA composites for use in cell-delivery applications in tissue engineering.

Methods: Human dermal fibroblasts were used as the cellular model. Collagen Type I was dissolved in acetic acid was mixed with HA to produce a final concentration of 4.0 mg/mL collagen with 16.0 mg/mL HA. Cells were suspended in the solution and the scaffolds were allowed to gel. Simulated body fluid (SBF) with 10% fetal bovine serum and 2% penicillin/streptomyocin was used as the experimental condition and standard Dulbecco's Modified Eagle Medium (DMEM) was used as the control. Both pure collagen and collagen-HA scaffolds were subjected to immersion in either SBF or DMEM at 37 °C and were characterized at days 0, 1, 3, 6 and 10. In one treatment group, collagen and collagen-HA scaffolds were placed in SBF for 3 days, followed by a "recovery" period in DMEM. Characterization of scaffolds included calcium content, compressive mechanical testing, cell viability, and histological analysis using H&E and von Kossa stains to localize cells and mineral.

Results: Calcium assay results (Fig. 1) indicated a significant increase ($p<0.05$) in calcium levels for collagen-HA scaffolds 3 days of SBF immersion compared to baseline collagen-HA scaffolds. Pure collagen scaffolds exhibited essentially no calcium deposition in either medium.

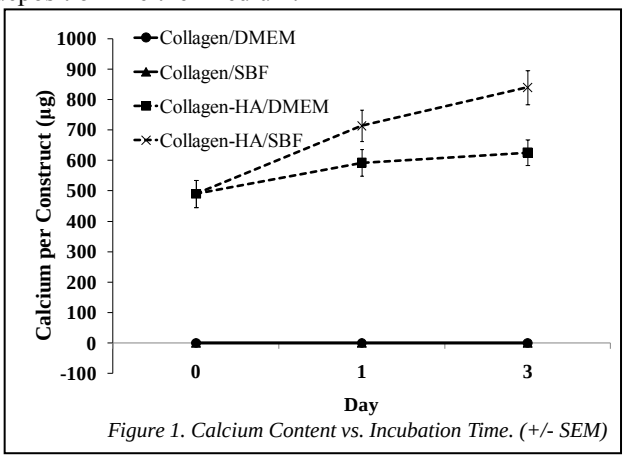

Figure 1. Calcium Content vs. Incubation Time. (+/- SEM)

The tangential modulus (Fig. 2) of collagen-HA scaffolds with SBF immersion increased over time and was higher than that of all other scaffolds and conditions.

Cell viability (90%) after 3 days of SBF incubation was comparable for all scaffolds to DMEM incubation. However, after 6 and 10 days, cells began to spread across the DMEM and recovered scaffolds, but not the SBF scaffolds. DMEM and recovered scaffolds also had higher live cell percentage (85%) than SBF scaffolds (25%).

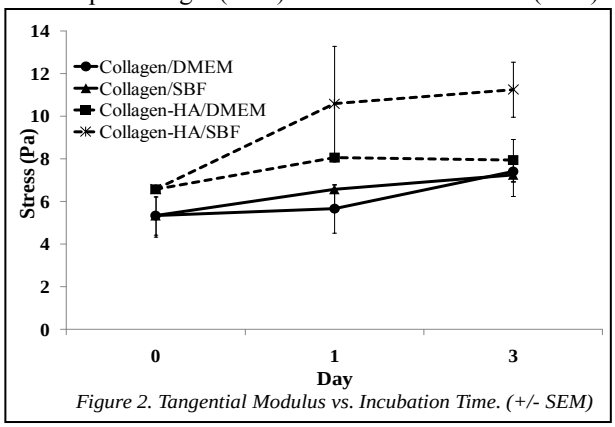

Figure 2. Tangential Modulus vs. Incubation Time. (+/- SEM)

Histology (Fig 3.) showed low levels of mineralization in pure collagen scaffolds and markedly increased mineralization in collagen-HA scaffolds, with the 3 day SBF scaffold containing the most mineralization. Mineralization began at the periphery and spread inward.

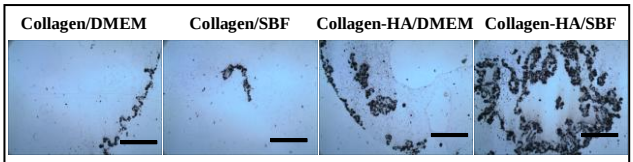

Figure 3. Histology – Day 3 von Koss Staining.

Conclusions: Incorporation of HA in collagen constructs facilitated more rapid mineralization of the matrix, as shown by from the calcium content and von Kossa staining. HA alone, however, had no significant effect on the mechanical properties of the material. SBF immersion was able to further increase calcium and phosphate levels in scaffolds, which also resulted in an improvement of mechanical properties. The incorporation of HA into collagen scaffolds did not adversely affect cells, however prolonged SBF incubation (more than 3 days) was shown to be toxic and induced cell death. Adverse effects of SBF on cell growth and viability could be avoided by subsequent recovery in DMEM.

The ability to rapidly mineralize protein matrices containing embedded cells may have utility in stabilizing engineered tissues, and is of particular interest in dental and orthopaedic engineering. HA integration and SBF immersion protocols can be further modified for other tissue engineering applications. Mineralization can improve mechanical properties as well as biocompatibility and biological integration of engineered tissues.

References: 1. Tan R. J Mater Sci Mater Med. 2009;20(6):1245-53. 2. Al-Munajjed AA. J Biomed Mater Res B Appl Biomater. 2009;90(2):584-91. 3. Kokubo T. Biomaterials. 2006;27(15):2907-15.