Novel Silk-Calcium Phosphate Ceramic Scaffolds for Bone Tissue Engineering

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Statement of Purpose: Critical-size bone defects are incapable of spontaneous healing and often require surgical intervention. The increasing number of skeletal injuries each year has motivated the fabrication of tissue engineered bone grafts as an alternative to autografting and allografting. Silk, a natural, biocompatible, bioresorbable polymer, has been united with calcium phosphate (CaP) ceramic via novel processing techniques to generate a bone graft with high compressive strength. Specifically, mechanical modeling has led to the design of a unique ceramic reinforcement component that can be embedded within a silk matrix to create a composite structure with robust mechanical properties. Since the ceramic component is completely embedded in silk, the graft provides a complete interface between silk and native bone across 100% of its surface area. Silk exhibits excellent osteoconductivity and promotes osteogenesis and defect closure through osteoinductive delivery of bone morphogenic proteins[1]. In addition, the central ceramic component does not require a high degree of porosity for initial cell ingrowth, which allows it to withstand higher loading forces than scaffolds comprised solely of CaP ceramic.

Methods: Three novel silk-based methods were developed to generate CaP ceramic parts (patent pending). Silk solution was first extracted from *Bombyx mori* cocoons[2]. Two of these methods involve the dissolution of CaP powders (Fisher Scientific, Pittsburg, PA) in an aqueous silk solution at various CaP/silk weight ratios. The mixtures were cast in silicone molds (Smooth-On Inc., Easton, PA) and either freeze-dried or treated with mild heat. The third method involves the use of a silk powder that was formed by freeze-drying silk solution into foam scaffolds and then blending them to create a re-dissolvable silk powder. The CaP and silk powder were mixed in varying CaP/silk weight ratios, dissolved in water, cast in silicone, and heat-treated. Green body ceramics were sintered at 1300 or 1400°C for 3 hours in a Lindberg Blue-M Tube furnace. The resulting sintered ceramic geometries were embedded in a silk sponge matrix via freeze-drying of silk solution around the ceramics parts. Total scaffold porosity was determined using mercury intrusion porosimetry. Pore size and interconnectivity in both the silk and ceramic matrices were analyzed with SEM imaging (Zeiss, Harvard CNS/NNIN). Compression testing was performed to assess the dependence of mechanical strength on porosity and macrostructure. Drug release kinetics, as relating to silk foam concentration, were analyzed by release profiles of various molecular weight FITC-Dextrans (Sigma-Aldrich). To assess cell distribution and viability within the silk and CaP matrices, hMSCs seeded on the scaffolds were stained and imaged with confocal microscopy.

Results: Mechanical testing of CaP ceramics scaffolds resulted in compressive strengths of 125-130MPa, which is approximately 76% the strength of human cortical bone (about 170MPa). In addition, since the silk solvent or silk powder used to generate the CaP parts acts as a sacrificial polymer during sintering, increased CaP porosity resulting from a decreased CaP/silk weight ratio leads to a lower scaffold compressive strength. Thus, the CaP/silk ratio can be modified to achieve a specific porosity range. Initial studies analyzing release profiles of FITC-Dextran from silk foams show an inverse relationship between silk concentration and release rate, with the highest percent group (12% w/v) releasing FITC-Dextran at a rate 3.5 times slower than the lowest percent group (2% w/v). Thus, the silk foam concentration can be tailored to control drug release profiles. In addition, SEM imaging has indicated that the silk foam concentration is inversely related to mean pore size; with concentrations over 8% w/v exhibiting significantly decreased pore size and cell viability. In vitro studies demonstrate excellent biocompatibility of the silk and ceramic materials.

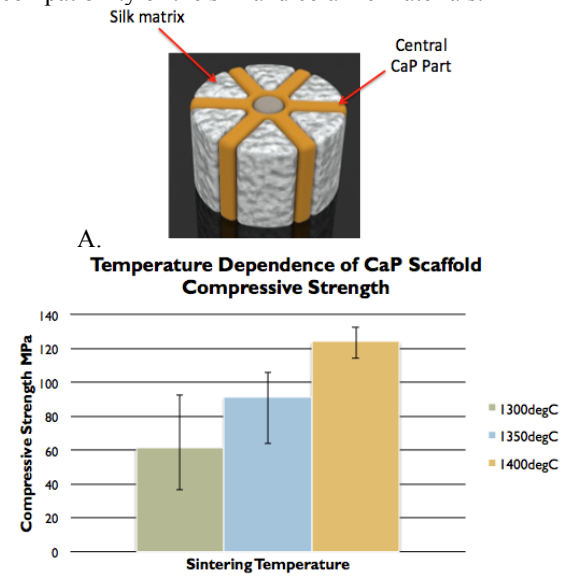

Figure 1A. Silk-CaP composite scaffold showing central ceramic component in a silk matrix. B. Dependence of CaP compressive strength on sintering temperature

Conclusions: Results from this study demonstrate that the novel silk-based CaP processing methods outlined above are effective in generating complex ceramic geometries. Moreover, these ceramics exhibit mechanical properties comparable to that of human bone. When embedded in a natural, biocompatible silk matrix these ceramics confer mechanical stability to the engineered graft while the encasing silk component interfaces with the native bone to induce healing. Tunable scaffold parameters include the degradation rate of both the silk and ceramic components, drug release rates from silk foam, pore size and pore volume of the silk and ceramic components.

References: 1. Kirker-Head, C. Bone 2007;42:247-255. 2. Altman, G. Biomaterials 2003;24:401-406.