Fabrication of High Strength Calcium Phosphate Scaffolds for Bone Repair Using Naturally Derived Silk Material

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Statement of Purpose: The field of bone tissue engineering is in need of simple yet reliable methods for creating resorbable bone grafts that are patient-specific yet mechanically competent.[1] Controllable factors in scaffold design must include three-dimensional shape, porosity, and mechanical strength to meet patient-specific defect geometries, encourage cell integration, and achieve a near perfect mechanical match at bone-graft interfaces.[2] We propose the combination of silk, a natural, biocompatible, bioresorbable polymer, with calcium phosphate (CaP) ceramic to generate high strength, complex geometry scaffolds via a series of novel processing techniques. Natural silk protein, when mixed with CaP particles, can be cured through beta sheet formation using mild heat, which in turn stabilizes the ceramic grains in a three-dimensional body prior to high temperature sintering. The use of silk for ceramic grain cohesion and consolidation allows for the fabrication of high density, complex 3-D scaffolds. During the sintering phase, the silk serve as a porogen leaving behind an interconnected pore network that is highly conducive to osteogenic cell ingrowth. Additionally, natural silk particles of defined size and shape can be included in the CaP/silk mixture to increase porosity through a 100% natural protein-ceramic composite using processing methods that are environmentally friendly and do not produce toxic byproducts. Furthermore, these silk processing methods can be used to produce sintered ceramics that are machinable, thus allowing for the production of 3-D patient-specific scaffolds.

Methods: Three patent-pending silk-based methods were developed to generate stabilized silk-CaP green bodies. Silk solution was first extracted from *Bombyx mori* cocoons.[3] Two of the silk-CaP methods involve the dissolution of CaP powders (Fisher Scientific, Pittsburg, PA) in an aqueous silk solution at various CaP/silk mass ratios. These mixtures are then cast in silicone molds or injection molded into cavities of pre-determined shape. The silk-CaP mixtures are then freeze-dried or treated with mild heat to stabilize the silk component to achieve a CaP green body of complex geometry. The third silk-CaP fabrication method involves the use of a soluble silk powder formed by freeze-drying aqueous silk solution and blending to form the un-degraded silk powder. For this method, the CaP and silk powders are mixed in various mass ratios, dissolved in aqueous medium, cast in molds, and heat-treated to stabilize. In any of the three silk-CaP methods, additional non-soluble silk macroparticles can be added to the CaP/silk solution to create high porosity scaffolds. Stable silk-CaP green bodies are then sintered at 1300°C or 1400°C for three hours in a Lindberg furnace to burn off the silk component and densify the ceramic grains. In some cases, sintered CaP blanks are machined into specified geometries using lathe cutting or CNC milling. X-ray diffraction analysis was performed on the sintered CaP material to determine phase transitions during heating (Scintag PAD-X, UMASS Lowell). Total scaffold porosity and 3-D architecture was analyzed using micro-computed tomography (X-Tek, Harvard CNS/NNIN) and confirmed with liquid displacement analysis. Pore size and pore interconnectivity were analyzed with scanning electron microscopy imaging (Zeiss, Harvard CNS/NNIN). Mechanical testing was performed to determine the compressive strength of sintered CaP ceramics made using various CaP/silk mass ratios. Cell viability and proliferation within the sintered CaP scaffolds was assessed using human bone marrow-derived MSCs. Analysis was carried out using Alamar Blue proliferation assay and imaging with confocal microscopy.

Results: Mechanical testing of CaP ceramics scaffolds resulted in compressive strengths as high as 155MPa and compressive moduli of 8-10GPa. In addition, the high strength scaffolds processed by this silk-based method are machinable with standard machining equipment. Furthermore, the total porosity of the sintered scaffolds can be controlled by varying the percent silk by mass in the green body, thereby allowing for the production of a range of porosity values between 5-60% for any bone regeneration model. Pore size also increases with percent silk in the green body, reaching an average of 80-100μm for 80% CaP/20% silk green bodies and up to 300μm for 60%CaP/40% silk with additional silk macroporogen particles. In vitro studies demonstrate excellent biocompatibility and cellular conductivity of the final sintered ceramic material.

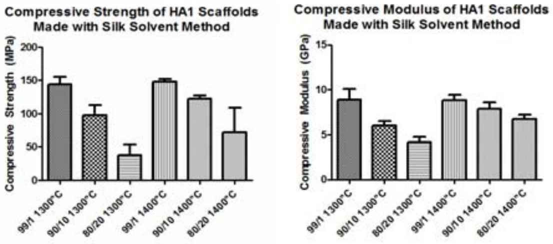

Figure 1. Compressive strength of sintered hydroxyapatite scaffolds at 1300°C and 1400°C with varying percent silk porogen material by weight.

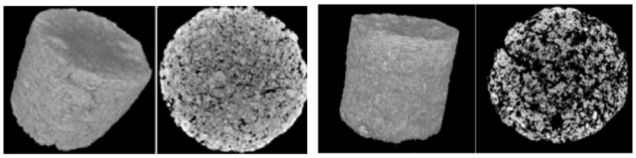

Figure 2. Micro-computed tomography images of a sintered ceramic produced from a green body of 80% CaP/20% silk (left), and a higher porosity ceramic produced by incorporating silk macroparticles (right).

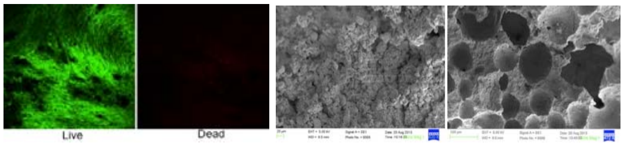

Figure 3. (Left) Confocal microscopy images of healthy, confluent MSCs proliferating on hydroxyapatite ceramics produced using 20% silk by mass (shown at 14 days post-seeding). (Right) SEM images of sintered ceramics formed from 60% CaP/40% silk without silk particles and with silk particles showing large pore sizes (scale bars: 10μm, 100μm).

Conclusions: Results from this study indicate that these novel silk-based CaP processing methods are effective in generating high-strength, biocompatible calcium phosphate ceramic scaffolds. Using only aqueous-based silk protein processing protocols, a wide range of material properties can be achieved for the end ceramic scaffold. Moreover, these scaffolds exhibit exceptional compressive strengths matching that of load-bearing cortical bone (150-160MPa). Compared to most commercially available calcium phosphate cements (30-60MPa), the silk fabricated scaffolds fabricated have the potential to serve as direct bone substitute under high load conditions.[3] The higher porosity scaffolds (60-70%), created by adding large silk particles as macroporogens, while mechanically weaker, can still match the strength of trabecular bone in non-load bearing bone regions. The additional porosity of these particular scaffolds provides a greater advantage in these high turnover areas because they allow for more robust cell integration and proliferation in bone regions where rapid regeneration is desired. Thus, these methods have potential application for a very broad range of bone replacement needs.

Significance: The addition of silk to a ceramic material prior to high temperature sintering is ideal not only because of the non-toxic nature of the silk itself and its environmentally friendly processing, but also because of the inherent cohesiveness of the silk. This proteins acts to binds and concentrates the ceramic grains creating a highly dense body, while later allowing for control of porosity during the burn-off phase. Depending on the method chosen to create the green body ceramic (e.g. silk solvent, silk powder, silk freeze-drying, silk macroporogens), and the percent silk material in the green body, the scaffold strength and porosity can be controlled to fit the specific needs of any bone defect. In addition, these silk processing methods can be combined with standard injection molding or machining processes to create scaffolds for patient-specific defect repair. Thus, silk-based ceramics engineering holds an exciting new potential for bone tissue repair.

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3. Altman G. et al. *Biomaterials.* **2003.** *24.* 401-416.

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