Calcium Phosphate Cement for Direct Writing of Tissue Engineering Scaffolds

Stefanie Crumlett, Neal K. Vail, Ph.D.
Southwest Research Institute, San Antonio, Texas

Statement of Purpose: We have developed a microextrusion technology to fabricate tissue-engineering scaffolds with controlled microstructure. We wish to fabricate strong calcium phosphate-based structures without the need for thermal post-processing. Therefore, we set out to develop a calcium phosphate cement (CPC) with custom curing and mechanical properties that could be used in the microfabrication process. CPCs are self-curing, osteoconductive hydroxyapatite precursors that find application in cranio-facial reconstruction (non-weight bearing) and are used as bone defect fillers (weight bearing). Commercial CPCs are putty-like materials capable of being shaped or injected into a defect where they cure and develop mechanical properties. CPCs are prepared "table-side," requiring mixing at time of use of a reactive powder precursor with an aqueous solution to promote curing, which can occur within minutes at physiologic conditions without causing thermally-induced necrosis. In our application, we require a CPC precursor formulation that is storage stable and supports microextrusion. Furthermore, the precursor should cure under specific conditions and have superior mechanical properties when cured. This paper discusses the development and characterization of a CPC-polymer composite with desired properties for our application.

Methods: We selected the following CPC chemistry [1]

$$\mathrm{ACP} + \mathrm{DCPD} \xrightarrow[\mathrm{H_2O}]{} \mathrm{HAp}$$

where ACP is an amorphous calcium phosphate with a Ca/P ratio of about 1.54, DCPD is dicalcium phosphate dihydrate, and HAp is hydroxyapatite. This chemistry was selected because the starting materials can be prepared by simple precipitation chemistry rather than rigorous thermal processes followed by material comminution. ACP and DCPD were synthesized by reported methods [2,3]. Compositions were determined by inductive coupled plasma spectroscopy. Crystal structure and crystallinity were determined by X-ray diffraction. Cements were formulated using 1:1 wt. ratios of ACP and DCPD then mixed with appropriate volumes of either water or dimethylsulfoxide (DMSO). Chitosan (Aldrich, low M_w), and sodium hyaluronate (HA, Genzyme, 560kDa) were formulated into the cement precursor to affect paste viscosity and cured mechanical properties. Rheological properties were determined in a parallel plate configuration (Rheometrics RFSII). Shear-dependent viscosity was measured over a frequency range of 0.1 to 100 rad/s. Modulus was measured at fixed shear over a time sweep of 60 minutes. Mechanical test specimens, 3mm dia. and 5mm tall, were prepared using a Teflon mold. Curing conditions were determined at 37°C and 50% relative humidity for a specified time. Compressive strengths were determined with a strain rate of 1mm/sec.

Results/Discussion: Mixtures of ACP and DCPD mixed in powder to liquid ratios (P/L, w/v) ranging from about 1.1 to 1.4 yielded a dough-like material. Curing resulted in compressive strengths of about 5-10MPa and conversion to HAp was confirmed by XRD. Higher P/L ratios (3.2 - 3.6) and similar handling consistencies were possible with DMSO as the liquid. Placing this DMSO-containing material (CPC precursor) in water resulted in solvent exchange and curing. Compressive strengths were typically two-times higher, probably due less porosity. The viscosity of water and DMSO formulations was similar over the P/L ranges studied and the materials were shear thinning. However, their zero-shear viscosities were much too high (> 10^6 P) for practical microextrusion.

Polymers were incorporated into the CPC precursor to affect their rheological properties for microextrusion. HA was added as a viscosity modifier, resulting in significant reduction of both the shear-thinning viscosity and storage modulus (see Figure 1). It is important to maintain a high storage modulus so that extruded filaments retain their shape following extrusion. We exploited the ionic interaction of anionic HA with cationic chitosan to increase the material stiffness. Figure 1 shows the effect of charge neutralization (i.e. [+]/[-]~1) of the HA with increasing chitosan on storage modulus. There is an order of magnitude increase in the modulus at low neutralization followed by only moderate increases with more neutralization. These precursor materials were subsequently placed in water for solvent exchange and curing. Both curing time and compressive strength increased. Conversion to HAp was also confirmed. The optimized CPC precursor was used to prepare sample structures by microextrusion.

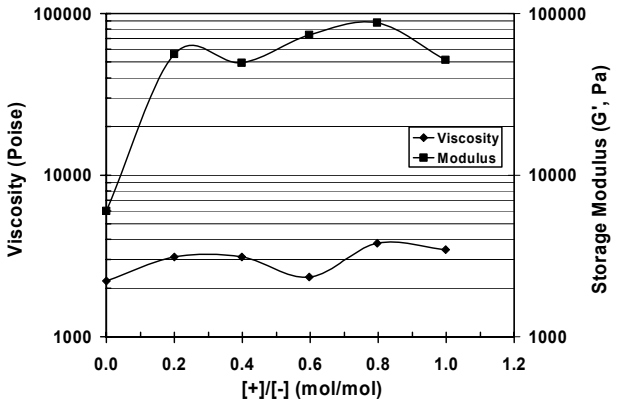

Figure 1. Effect of charge neutralization on CPC precursor viscosity and storage modulus.

Conclusions: A custom-curing calcium phosphate cement formulation with tailored rheological properties was developed for use with microextrusion technology to fabricate mechanically strong tissue engineering scaffolds.

References: 1. USP 5,650,176. 2. Li, et al., J Mat Sci Letters. 2003; 22:1015-1016. 3. USP 6,544,290.