Fabrication and Characterization of Calcium Phosphate Mineralized Collagen-GAG Scaffolds

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Statement of Purpose: It has been estimated that over 62 million tendon and ligament injuries occur every year. The failure rate for these injuries can be despairingly high, such as 94% in the case of rotator cuff injuries.[1] One possible cause for these failures is the inadequate recapitulation of the interface between the two mechanically and chemically different tissues, like bone and ligament. We are developing a multi-compartment regenerative scaffold designed to mimic the distinct tendinous and osseous compartments as well as the continuous interface. Such a scaffold could induce the physiological wound healing processes in critical defects, while maintaining the mechanical and chemical properties inherent in the native tissue. The present project is developing fabrication and characterization strategies for a calcium phosphate mineralized collagen-GAG (CGCaP) scaffold and a multi-compartment collagen-GAG (CG) scaffold with a gradient in mineral content, mimicking the bone-tendon-insertion (BTI) site.

Methods: CGCaP scaffolds are fabricated via lyophilization from a suspension of phosphoric acid, collagen, chondrotin sulfate, calcium hydroxide, and calcium tetrahydrate.[2] In the case of the multi-compartment scaffolds tendon to bone insertion scaffold, an additional liquid phase co-synthesis step was taken to layer CG and CGCaP suspensions prior to lyophilization.[3] By controlling the freezing temperature and rate during lyophilization, different pore structures have been identified. Additional crosslinking steps can further strengthen the scaffold.[3]

Stereology and two modified chemical assays, a 1,9-dimethylene blue assay and a hydroxyproline assay, were employed to determine scaffold pore size and mineral content.[4,5] The mineral content was further characterized with powdered x-ray diffraction to determine the mineral phase of the precipitated calcium phosphate.[2] X-ray microtomography was used as a nondestructive alternative to determine pore size, porosity, pore interconnectivity, and mineral distribution. Scaffold permeability and mechanical characterization is being assessed via conventional approaches.[7] Ultrasound elastography approaches are being used to determine local mechanical and permeability measurements in composite scaffolds.[6]

Results: Results from stereological analysis (Fig. 1) show an open-pore structure with a mean pore size of 134±20μm. 1,9-dimethylene blue and the hydroxyproline assays determined scaffold calcium phosphate mineral content to be 39.5±4.4 wt%, consistent with physiological bone.[2] Additionally, through powder x-ray diffraction, the phase of the calcium phosphate mineral content was found to be roughly 25% brushite and 75% monotite (Fig. 1); ongoing work is exploring hydrolysis steps to generate apatite-CGCaP scaffolds. X-ray microtomography has confirmed the calculated pore size, and has determined scaffold pososity to be 94%. Initial results from ultrasound elastography measurements have shown expected strain measurements for in-plane and out-of-plane axes (Fig 2) during compression of the (low density, open-cell foam) CG scaffold.[6]

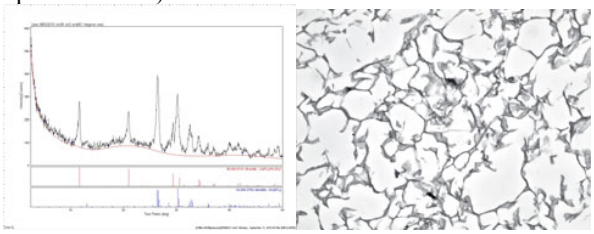

Figure 1. Powder x-ray diffraction data identifying brushite and monotite (left); stereology image (right).

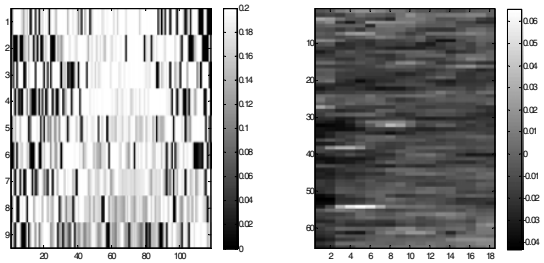

Figure 2. Local strain map parallel to compression (left) and the corresponding strain map perpendicular to compression (right) obtained from ultrasound elastography, with intensity indicating strain.

Conclusions: We have developed consistent fabrication strategies to create mineralized CG scaffolds with control over scaffold microstructure (pore size/shape, porosity) and composition. On-going projects are focusing on the characterizing the bulk permeability and mechanical properties in compression. These measurements will then be used to compare the permeability and mechanical properties measured locally with ultrasound elastography; this tool will be particularly useful for local characterization of interfacial scaffolds. Additional projects are addressing fabrication[3] and subsequent characterization of multi-compartment scaffolds mimicking the BTI site. It is also expected that these characterization techniques will provide fundamental knowledge regarding physiological interfaces.

References:

[1]Li X. Nano Lett. 2009;9:2763-2768.
[2]Lynn A. J Biomed Mater Res A. 2010;92:1057-1065.
[3]Harley B. J Biomed Mater Res A. 2010;92:1066-1077.
[4]Barbosa I. Glycobiology. 2003;13:2763-2768.
[5]Samuel C. Meth Molc Bio. 2009;446:1-13.
[6]Harley B. Chem Eng J. 2008;137:102-121.
[7]Kalyanam S. J Biomech Eng. 2009;131:081005.