Prediction of Collagen and Glycosaminoglycan Content by Acoustic Microscopy

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Statement of Purpose: Functional tissue engineering of articular cartilage is rapidly advancing as a technique to develop regenerative and reparative treatments for cartilage degeneration and osteoarthritis. Tissue-engineered constructs are often developed by use of a combination of cells, polymer scaffolds, biochemical mediators, and mechanical cues, and are then evaluated by a variety of histological, biochemical, and mechanical techniques. However, testing methods are often time-consuming and destructive to the construct, creating the need for real-time, nondestructive measurement techniques. High-frequency ultrasound has been demonstrated as a technique sensitive to mechanical and biochemical properties of cartilage (*1*). Therefore, ultrasound has the potential to nondestructively measure the concentration of extracellular matrix (ECM) molecules and assess the quality of tissue-engineered cartilage during development. The objective of this research was to investigate the correlation between attenuated reflection coefficient and the concentration of collagen and glycosaminoglycan (GAG) in enzymatically degraded bovine articular cartilage, with implications for evaluating the quality of tissue-engineered cartilage during development.

Methods: Bovine articular cartilage explants were digested in type II collagenase for 1, 2, 3, or 4 h. Biochemical assays and histological staining for collagen and proteoglycan were used to characterize samples at each degradation time point. GAG and soluble collagen content were quantified with the commercial Blyscan and Sircol assays (Biocolor Ltd, Newtownabbey, Northern Ireland*), respectively. For histological staining, thin (10 μm) slices near the top surface and middle of the sample were obtained at each time point. Masson's trichrome stained collagen blue and Safranin-O stained proteoglycans red/orange. Acoustic microscopy with a 50 MHz acoustic transducer was used to image samples and to extract information about changing tissue properties and their relationship to high frequency ultrasonic wave propagation properties. Orthogonal, linear regression analysis was performed on both GAG and collagen concentration data with respect to attenuated reflection coefficient data.

Results: Digestion of cartilage explants with collagenase resulted in a significant loss of tissue mass resulting from the digestion of both collagen and proteoglycan. Acoustic images of the explants after digestion are consistent with a loss in mass. The images demonstrate an increase in signal reflection with the decrease in mass (data not shown). The attenuated reflection coefficient was measured at each digestion time point and was compared with collagen and GAG content of the samples. Linear regression analysis resulted in statistically significant correlations between ECM content and attenuated reflection coefficient (Figures 1 and 2).

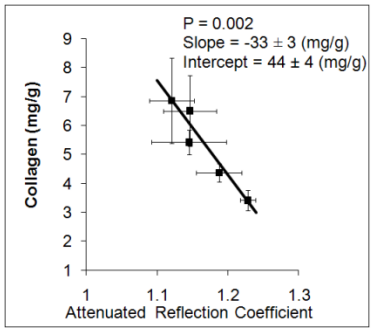

Figure 1. Relationship between collagen concentration and attenuated reflection coefficient.

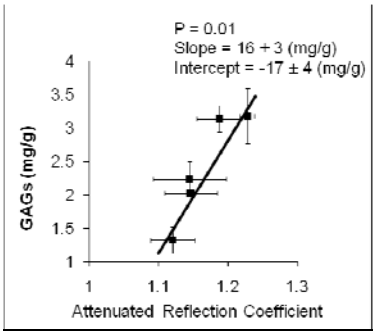

Figure 2. Relationship between GAG concentration and attenuated reflection coefficient.

Conclusions: Attenuated reflection coefficient is directly correlated with the concentration of both GAG and collagen in bovine articular cartilage. These results have implications for the use of acoustic microscopy to nondestructively evaluate the development of tissue engineered cartilage.

Continuing work focuses on the ultrasonic evaluation of tissue engineered cartilage during development in a dynamic compression bioreactor. Ultrasonic measurements can be obtained without removing the construct from the reactor. Bovine chondrocyte-seeded poly(ethylene glycol) hydrogels will be incubated in the bioreactor under intermittent cyclic load. Tissue development will be monitored using ultrasonic microscopy and mechanical testing. Histological and biological assays will be used to quantify ECM development and correlated with ultrasonic and mechanical data.

Reference: 1. M. A. Rice, K. R. Waters, K. S. Anseth, *Acta Biomater* **5**, 152 (2009).

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