INTRODUCTION Osteoarthritis (OA) is a progressive disease of articular cartilage which affects more than 20 million Americans. While novel physical and pharmacologic modalities are currently being intensively explored towards attenuating the progression of this disease, the ability to detect early osteoarthritic changes is crucial for optimal development and application of these interventions. Because anatomic changes in early OA are subtle at best, one promising approach to early detection is to identify molecular alterations in the cartilage matrix. Such changes include collagen breakdown and proteoglycan degradation. Accordingly, we investigated an in vitro model of OA using Fourier transform infrared imaging spectroscopy (FT-IRIS) in order to demonstrate the sensitivity of this method to cartilage matrix alterations. Previously, FT-IRIS has been used to evaluate the quantity, distribution and orientation of collagen, and quantity and distribution of proteoglycan in normal cartilage [1,2]. In the current study, we used a well-characterized hollow-fiber bioreactor (HFBR) system to produce hyaline cartilage from chick sternal chondrocytes under control conditions [3] as well as with chondroitinase (CA)-supplementation of tissue culture medium.

METHODS Tissue Production: Chondrocytes were obtained from the distal half of the sterna of Day 16 chicken embryos by collagenase digestion. After appropriate resuspension, 3 x 10^7 cells were inoculated into bioreactors constructed from glass tubing (inner diameter 4 mm, length 60 mm) and through which six porous polypropylene hollow fibers (inner diameter 330 microns, 0.2 mm pores, wall thickness 150 microns) were extended longitudinally and potted at either end of the glass tube using silicon rubber. The HFBR’s were perfused with medium and maintained in a 5% CO2, 95% air incubator. Control (CTL) bioreactors received fresh tissue culture medium (TCM), while the CA group received TCM supplemented with CA for a total period of 10 days during 4 weeks of growth, at which time biochemical and FT-IRIS analysis were performed. Biochemical Analysis: Cartilage tissues corresponding to the FT-IRIS imaging sections was subjected to biochemical analysis. Percent water content was determined by dessication. Samples were then pulverized and digested in a solution containing 300 mg/ml papan. Chondroitin sulfate was determined using the dimethylmethylene blue dye binding colorometric assay. Aliquots of the papain digest hydrolyzed with 6 N HCl, and the hydroxyproline (HP) content of the sample was determined from absorbance at 557 nm. Collagen content was derived from the HP content. FT-IRIS: Tissues were embedded in OCT embedding media and cryosectioned directly onto barium fluoride windows for FT-IRIS analyses. Both experimental groups, CTL and CA, were comprised of four bioreactors. A BioRad (Cambridge, MA) UMA 300A FTIR microscope with an FTS-60A step-scanning FTIR spectrometer and a 64x64 MCT FPA detector (Stingray Imaging Spectrometer) was used to acquire FT-IR data at 16 cm^-1 resolution under N2 purge. Information on collagen and proteoglycan content and distribution was obtained from a 400 x 400 µm^2 region, resulting in 4,096 individual spectra for each sample. OCT was spectrally subtracted from each image, and the type II collagen amide I and amide II absorbances were monitored in the 1590-1720 and 1480-1490 cm^-1 infrared regions respectively. Images based on the integrated area of this absorbance were created to determine type II collagen quantity and distribution. The proteoglycan (PG) absorbance that arises from the sugar moiety was monitored in the 980 – 1145 cm^-1 region. The integrated area of this absorbance was ratioed to the amide I collagen absorbance to obtain relative quantity and distribution of the PG component (PG/AmI). The ratio of the amide I to amide II absorbance (AmI/AmII) was utilized as an indicator of collagen quality. Data was analyzed using Iysys software (Spectral Dimensions Inc, Olney, MD).

RESULTS Biochemistry: Chondroitinase treatment resulted in a significant reduction in chondroitin sulfate content (Control: 37 +/- 3%, CA: 17 +/- 4%; p < 0.001). Collagen content per dry weight did not change significantly. These results are consistent with the known actions of chondroitinase.

FT-IRIS images based on collagen content (amide I + amide II integrated area) reveal changes in the morphology of the tissue and collagen distribution with CA treatment (Figure 1A,C). In the control tissue, the edges are sharply defined by collagen, in contrast to the CA tissues where the concentration of collagen is heterogeneous at the edges, and apparently reduced overall. In addition to the reduction in collagen distribution, a change in the collagen environment was evidenced by a reduction in the AmI/AmII with CA treatment (1.35 +/- 0.13 vs 1.6 +/- 0.0, p = 0.008). The PG concentration (PG/AmI) was significantly reduced in the CA tissue compared to control (0.92 +/- 0.13 vs 0.97 +/- 0.12, p < 0.001). This difference was manifested as an overall reduction throughout the tissue (Fig. 1B, D).

DISCUSSION FT-IRIS is a powerful technique which can assess matrix quality in a variety of connective tissues. The present study addresses the hypothesis that FT-IRIS is sensitive to molecular alterations in cartilage matrix. HFBR-derived tissue is typical hyaline cartilage with abundant collagen II mRNA expression, and hence is a relevant model for an initial study addressing this hypothesis. We found that indeed, FT-IRIS results correlated qualitatively with biochemical determination. We note that the biochemical evaluation revealed a more marked decrease in PG than did the FT-IRIS technique. We attribute this to an underestimate of PG in the FT-IRIS data resulting from the use of OCT as an embedding medium; we performed correction for the OCT absorbance overlying the PG absorbance using a conservative spectral subtraction technique which ensured that the FT-IRIS PG determination was not artifactually low. One surprising finding was a statistically significant difference in the ratio of the amide I to amide II spectral bands in the CTL and CA groups, likely indicating an alteration in collagen at the molecular level which occurred in the absence of a biochemically-detectable difference in the overall collagen concentration. This may reflect changes in the collagen environment that arise from altered proteoglycan content. These data demonstrate the utility of FT-IRIS in the evaluation of altered cartilage matrix. The ability to simultaneously obtain information on collagen and proteoglycan content, distribution, and environment has the potential to significantly aid in the development of novel therapeutics for cartilage disorders.

REFERENCES: