Imaging the Local Biochemical Content of Native and Injured Intervertebral Disc using Fourier Transform Infrared Microscopy

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INTRODUCTION:

Alterations to the biochemical composition of the intervertebral disc (IVD) are hallmarks of aging and degeneration[1,2,3], however these changes have largely been quantified through methods that assess the composition of bulk tissue. It is currently unknown how the local biochemical composition of IVD tissue is affected by degeneration. Previous studies have used Fourier transform infrared (FTIR) microscopy to measure the local proteoglycan and collagen composition of articular cartilage and other cartilaginous tissues[4], however this technique is largely unexplored for the IVD. Here we use FTIR microscopy to image the local biochemical content of sheep IVDs before and after induced degeneration from an annulotomy injury. The objective of this study was to determine the feasibility of imaging native and degenerated IVD with FTIR microscopy, and compare the FITR method with standard histological imaging.

METHODS:

Histology blocks were obtained from intact (Pfirrmann grade 1) and degenerated (Pfirrmann grade >2) IVDs from a previous *in vivo* study in which 4 skeletally mature female Finn sheep were used with Institutional Animal Care and Use Committee approval. The sheep underwent a surgical procedure following a previously described method[5], where IVDs from L1 to L6 of the lumbar spine were exposed using a lateral access approach. IVDs were either left intact or injured via a 3 cm x 1 cm box annulotomy and partial nucleotomy to induce degenerative changes *in vivo*. After 6 weeks the spines were harvested, the IVDs were isolated from surrounding tissue, and processed for histology. IVDs were sectioned in the mid-coronal plane onto either infrared-transparent barium fluoride slides (Spectral Systems, Hopewell Junction, NY) for FTIR microscopy, or glass slides for Safranin-O histology. FTIR samples were loaded into a Hyperion 2000 FTIR microscope (Bruker, Billerica, MA) and FTIR spectra were obtained from 250µm x 250µm squares across the sample, collected in a grid where each measurement was acquired 250µm apart. The peak area of the amide I, amide II, sulfate and sugar peaks were analyzed using a custom MATLAB code (Mathworks, Natick, MA) that referenced literature wavenumbers for peak widths[6]. Peak area maps of intact and injured IVDs were compared to validate the FTIR imaging technique against known changes in IVD composition with degeneration.

RESULTS:

A total of 1 intact and 6 injured IVDs were used to collect FTIR spectra maps. IVDs sectioned in the mid-coronal plane yielded FTIR spectra with distinct amide I, amide II, sulfate, and sugar peaks (Figure 1A). Amide I and II peaks are typically associated with collagen while sulfate and sugar peaks are associated with proteoglycans, however multiple peaks were investigated due to the overlap in FTIR signatures between collagen and proteoglycans (Figure 1A). Peak area maps for the amide I, amide II, and sulfate peaks all showed a similar signature in the annulus fibrosus (AF), correlating well with known collagen content (Figure 1B). The regions with high amide I, amide II and sulfate peak areas are part of the outer AF as seen in Safranin-O histology (Figure 1C). Sugar peak area maps correlated well with known proteoglycan-rich regions of the IVD, and showed a unique signature in the nucleus pulposus (NP) with much lower peak areas in the AF. Amide I and sugar peak area maps showed the intact IVDs have greater collagen and proteoglycan content than the injured IVDs (Figure 1C). When quantified, the average sugar peak area of intact IVDs was higher than injured in both the AF and NP (Figure 1D).

DISCUSSION:

The objective of this study was to explore FTIR microscopy as a method to determine the local biochemical composition of the IVD. FTIR microscopy was able to parse out the collagen and proteoglycan content of intact and injured IVDs with high spatial resolution. Traditional histological imaging using Safranin-O stain showed distinct NP and AF regions, while FTIR microscopy enabled visualization of the collagen and the proteoglycan-rich regions of the IVD. Specifically, amide I peak area maps were intense in the outer AF, while sugar peak area maps showed a large proteoglycan-rich region that encompassed both the NP and inner AF. Comparing intact and injured IVDs validated the applicability of FTIR microscopy for the IVD, where large decreases in proteoglycan were seen with degeneration but little change in collagen content, consistent with previous literature[2]. FTIR microscopy reduces the need to set aside samples for bulk biochemical analyses since histology blocks can be sectioned onto barium fluoride slides. Future work will perform traditional biochemical methods in parallel with FTIR microscopy to correlate spectral peak areas to absolute collagen and proteoglycan concentrations.

SIGNIFICANCE:

Quantifying the local distribution of proteoglycan and collagen content enables researchers to better understand the development, health and disease progression of the IVD.

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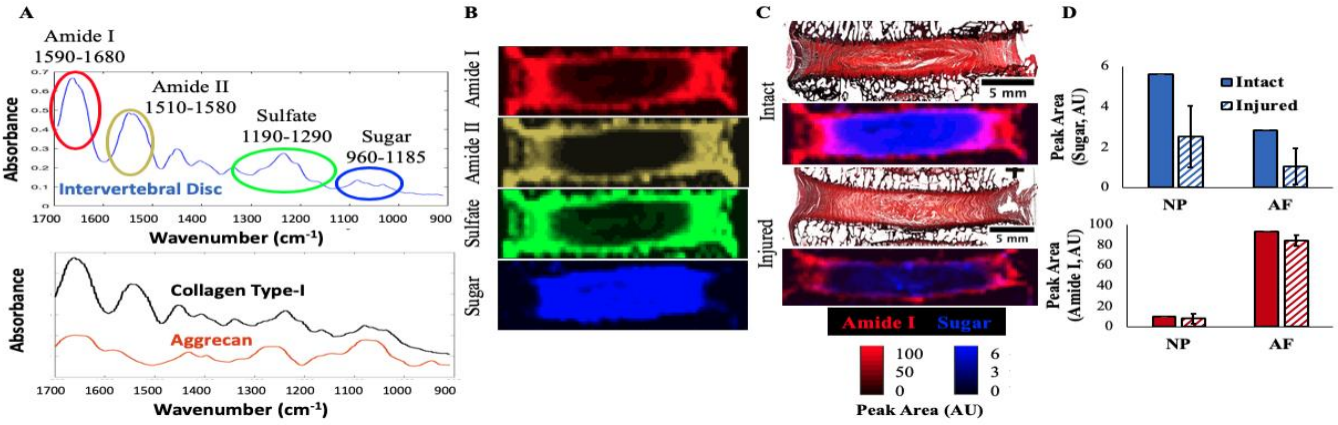

Figure 1: A) Representative FTIR spectra obtained from a 250µm x 250µm area of an IVD, as well as the FTIR spectra for pure collagen type-I and aggrecan. The peak area of the amide I, amide II, sulfate and sugar peaks was calculated at each raster point across the IVD. **B)** Representative maps of the peak areas for an intact IVD. **C)** Safranin-O histology and merged maps of amide I and sugar peak areas for intact and injured treated IVDs. **D)** Peak area measurements for the amide I and sugar peaks in both the AF and NP for intact and injured treated IVDs, n = 1-6, error bars are ± STD.