Identification of Biomolecular Changes in Intervertebral Disc Tissue Using Fourier Transform Infrared Spectroscopy

Sumona Sarkar,[1] Christopher Massey,[1] Michele Marcolongo[1]
[1]Drexel University, Philadelphia, PA USA

Introduction. Intervertebral disc (IVD) mechanics are primarily governed by the osmotic pressure in the nucleus pulposus (NP) and the fibrous structure of the annulus fibrosus (AF) [1]. Three mechanically important constituents of IVD tissue are proteoglycans (PG), collagen (COL) and elastin (ELA). Alterations in the major biochemical constituents of the IVD have been shown to coincide with age and disc degeneration and can subsequently alter the discs' ability to support load [2,3,4]. Fourier transform infrared (FTIR) spectroscopy may provide a powerful tool to monitor changes in IVD constituents with aging and degeneration [5]. In this study, FTIR spectra for each of the major components of IVD tissue were gathered and investigated at varying ratios, the effect of digestive enzymes on the tissue were studied, and the FITR spectra of IVD of varying degenerative grade were examined.

Materials and Methods. Elastin, collagen I, and chondroitin sulfate (CS), a major constituent of PGs, were obtained in their pure forms and analyzed at varying ratios using FTIR. Distinct peaks in these components were determined and their peak height ratios examined. IVD tissue specimens were isolated from the AF and NP of a 79yo, male spine (L3-L2). Samples were investigated after no treatment and after 1hr soaks in increasing concentrations of pancreatic elastase. Annulus samples were also isolated from calf spines and subjected to collagenase, chondroitinase ABC and elastase treatments. Calf IVDs were chosen since they are generally in a consistently normal state and thus can be selectively degenerated. Samples were lyophilized and prepared into pellets with KBr and analyzed using transmission mode FTIR and the OMNIC software package (resolution = 2, number of scans = 128). NP and AF samples were also gathered from L1-L2 IVDs of human cadaver spines aged 15, 58, and 79 years old with increasing levels of degeneration and analyzed similarly with FTIR

Results. Peaks for COL and ELA were detected in the Amide II region (1600-1585cm^{-1}) with peaks appearing at 1552 and 1540cm^{-1} respectively. CS had no detectable peak in this region but did demonstrate a shared peak with COL in the Amide III/Sugar region (1100-1050cm^{-1}) with peaks at 1082cm^{-1} for COL and 1067cm^{-1} for CS. Digestive treatments targeting COL, ELA, and PGs of the AF resulted in shifts in peak wavenumber corresponding to a loss in the targeted component. Serial digestion of AF and NP tissue with elastase resulted in a progressive change in peak wavenumber in the region 1588-1474 cm^{-1} as seen in Fig 1. Spectra from the NP and AF samples of discs with varying grades of degeneration and age were analyzed and the ratio of peak heights for the Sugar (1036cm^{-1}) and Amide II (1543cm^{-1}) regions determined. With age and degeneration, the Sugar/Amide II peak height ratio decreased in the NP and in the AF (Fig 2). In order to better understand the relationship between the ratio of IVD components and their subsequent FTIR spectra, compounds with varying ratios of pure ELA, COL and CS were investigated and a linear relatiohsip between CS:(ELA + COL), (PG: Protein) and Peak Height Ratio of the Sugar/Amide II regions was determined ($R^2 = 0.837$),

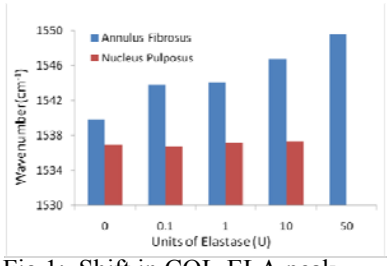

Fig 1: Shift in COL-ELA peak with elastase digestion

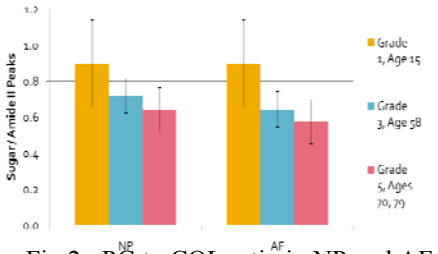

Fig 2: PG to COL ratio in NP and AF

Discussion. Peaks in the Amide II, Amide III and Sugar regions are modified in terms of peak height ratio as well as peak wavenumber with changes in component ratio, degeneration level, and digestion level. Systematic treatment of IVD tissue with elastase indicates that changes in ELA content may be monitored using FTIR by investigating Amide II peak position. Changes in PG to total protein content may be quantitatively measured observing changes in peak height ratio of the Sugar to Amide II peak as indicated by the linear relationship seen when varying pure component ratios. Trends were observed when comparing peak height ratio to IVD degenerative grade where a decrease in the sugar/protein ratio is seen with increasing degeneration. This correspond to trends found in literature where a loss of overall PG content, as well as an increase in COL, is seen in the NP with aging, while the AF shows a loss in PGs with degeneration, but the overall COL content is believed to remain relatively constant [2,3].

Conclusions. This study shows that FTIR may be a useful tool in analyzing changes in IVD biomolecular makeup during disc degeneration. Understanding the correlation between biomolecular changes that take place in the IVD during degeneration and the associated mechanical failures may provide an avenue for more targeted early therapeutic interventions.

References. 1. Adams MA et al., Spine, 31, 2151, 2006; 2. Olczyk K, Gerontology, 38, 196, 1992; 3. Olczyk K, Z Rheumatol, 53, 19, 1994; 4. Olczyk K, Folia Histochem Cytobiol, 32, 41, 1994; 5. Camacho, NP et al., Biopolymers, 62, 1, 2000.