Assessment of an In Vitro Animal Model for Intervertebral Disc Degeneration Using MR Elastography

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Introduction: Low back pain (LBP) is a very costly and prevalent health disorder in the U.S., resulting in total costs exceeding $100 billion per year.[1] In fact, its reported that up to 85% of people will experience LBP in their lives.[2] One of the most common causes of LBP is degenerative disc disease (DDD), which has been shown to precede LBP and several other disorders in the spine associated with LBP.[3] DDD is a complex cascade of biological and structural changes in the intervertebral disc (IVD) caused by altered mechanics and load distribution of the disc. There are many techniques that have been developed to characterize disc degeneration, but there is no way to directly assess the material properties of the IVD *in vivo*. It has been shown that the nucleus pulposus within the disc undergoes significant changes in shear modulus, even with early stage disc degeneration.[4] Magnetic resonance elastography (MRE) is a sensitive, phase contrast-based imaging technique for non-invasively mapping the mechanical properties of tissues.[5] Previous studies have demonstrated the feasibility of using MRE in the IVD *in vitro* to estimate the nucleus pulposus stiffness.[6,7] The *purpose* of this study is to determine if MRE is capable of detecting a stiffness change in the intervertebral disc in an *in vitro* animal model of enzymatically-induced disc degeneration. The target audience of this research is MRI scientists involved in developing spinal imaging methods, radiologists involved in spinal imaging, clinicians involved in managing patients with low-back pain and disc-related spinal disorders, and basic scientists investigating DDD.

Methods and Materials: (1) Intervertebral Disc Specimens. Three spinal motion segments (T12/L1, L2/L3, and L3/L4) of a goat lumbar spine were removed with musculature and entire IVD intact. All posterior elements of the motion segment were removed to increase flexibility of the each specimen, such that each motion segment consisted of partial upper and lower vertebral bodies and an intact IVD. (2) *In Vitro* Disc Degeneration Model. According to Roberts et al, trypsin or papain can be used to create an explant model for disc degeneration in bovine caudal discs.[8] The L2/L3 goat IVD was injected with ~100 µL of trypsin at 20 mg/mL into the nucleus to induce disc degeneration, as reported in the literature. The other two discs served as normal controls. (3) Mechanical vibration. A piezoelectric mechanical driver was used to apply mechanical vibrations at frequencies in the 1-10 kHz range. The driver was positioned such that shear vibration was applied to the upper vertebral body of the motion segment while the lower vertebral body was fixed using a custom-built testing fixture. The specimen was put into a single-channel, 3-in diameter receive coil with the transverse, or axial, cross-section of the IVD parallel to the B_0 direction. The vibration direction was parallel to the B_0 direction and perpendicular to the spinal motion segment (fig. 1). (3) Wave imaging sequence. The disc specimens were imaged using a spin echo-based MRE sequence with the following parameters: 30 total cycles of 1000Hz motion-encoding gradients (2.4 Gauss/cm), motion sensitivity = 12.9µm/π, offsets = 4, 290/50-ms TR/TE, 8-cm FOV, one 8-mm slice, 256x96 matrix, 1 NEX. A standard 1.5T full-body MRI scanner (Signa 16X Software, GE Healthcare, Waukesha, WI) was used in the experiment. Motion encoding was done in the S/I direction, or along the direction of motion, in all scans. (4) Inversion algorithms. The resulting wave images were then masked, phase unwrapped, bandpass filtered (1-20 waves per FOV), directionally filtered (8 directions),[9] and processed using a local frequency estimation (LFE) inversion algorithm to provide maps of shear stiffness.[10] The average shear stiffness of the nucleus pulposus region within the IVD was measured with a circular ROI placed in the center of the disc as shown in the magnitude and stiffness map images (fig. 2).

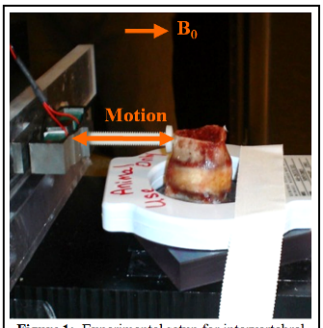

Figure 1: Experimental setup for intervertebral disc MRE with motion applied to the upper vertebral body using a piezoelectric driver while the lower vertebral body is fixed.

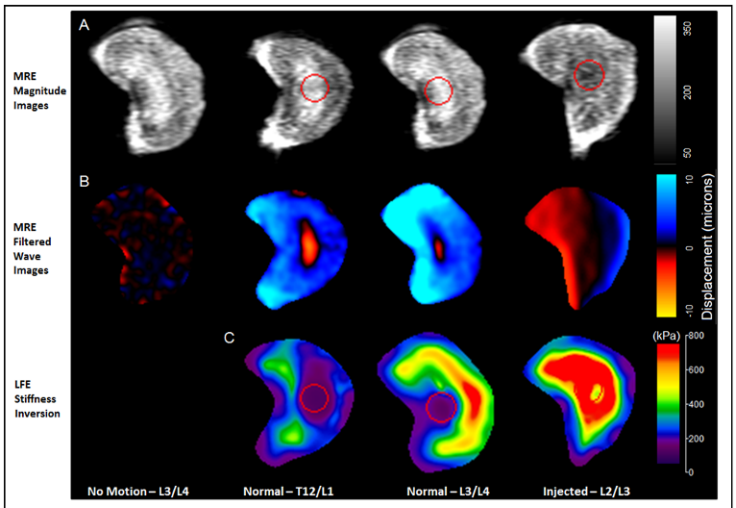

Figure 2: Top row (A): MRE magnitude images of intervertebral discs. Middle row (B): representative wave images in S/I encoding direction after filtering with a Gaussian bandpass filter with cut-offs of 1 and 20 waves/FOV. Color bar indicates the motion amplitude measured with MRE. Bottom row (C): Shear stiffness maps generated from wave images using LFE inversion algorithm. Color bar indicates shear stiffness in kPa. Red circles indicate ROI used for nucleus stiffness approximation.

Results and Discussion: As shown in Figure 2, shear waves were seen in axial cross-sections of both normal discs and the trypsin-injected disc. In the normal control discs, the shear wave propagation appears distinctly different in the annulus compared to the nucleus region of the disc, with the nucleus showing a much shorter wavelength as seen in the filtered MRE wave images (fig. 2B). The wave image for the no motion case shows very little, incoherent motion as expected (fig. 2B). The wave data, for all but the no motion case, was inverted using the LFE inversion algorithm to give a shear stiffness map for the transverse cross-section of the discs (fig.2C). Although the wavelength was too long in the annular region to reliably approximate stiffness, the nucleus showed an average stiffness of 89 ± 14 kPa and 133 ± 25 kPa in the T12/L1 and L3/L4 normal discs respectively. These nucleus stiffness values are both very near the range reported in literature for the healthy human IVD nucleus stiffness.[11,12] The trypsin-injected disc, L2/L3, showed a much longer shear wavelength in the nucleus (fig. 2B), and had a much higher average stiffness of 715 ± 145 kPa.

Conclusions: These initial results suggest MRE is capable of detecting changes in the nucleus stiffness in this explant model for enzymatically-induced IVD degeneration. Additionally, the data suggests MRE can differentiate the nucleus and annulus regions of normal intervertebral discs, but this difference may be lost with degeneration. According to Roberts et al, this model for disc degeneration could be used to test novel injectable materials for reversing degenerative changes. Based on this work, it appears disc MRE could be used to compare and monitor the effects of novel intervertebral disc treatments using this explant degenerative model.

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