Mechanical and Biochemical Characterization of the Degenerated Rabbit Intervertebral Disc by MRI

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INTRODUCTION: Mechanical behavior and biochemical composition are both important measures of the function of intervertebral discs [1] and the progress of degeneration [2]. MRI techniques have the ability to nondestructively quantify both mechanical and biochemical characteristics of discs. Displacement-encoded stimulated echoes with fast spin echo readout (DENSE-FSE) can quantify displacements within a tissue of interest with displacement precisions exceeding 8.8 μm [3]. Delayed gadolinium enhanced MRI of cartilage (dGEMRIC) allows for the quantification of glucosasminoglycan (GAG) concentration [4]. In this study, both of these MRI techniques are used to evaluate the mechanical and biochemical consequences of a puncture model of intervertebral disc degeneration [5, 6].

METHODS: Intervertebral discs (IVDs) between the 4th and 5th lumbar vertebrae (L4/L5) of eight (n = 8) New Zealand white rabbits were punctured with a 16-gauge needle under aseptic conditions and Institutional Animal Care and Use Committee approval. Animals were sacrificed after 28 days, and the L4/L5 and adjacent L3/L4 IVDs were isolated to include the full disc and approximately 3mm of the attached superior and posterior vertebral bodies. For imaging experiments, IVDs were mounted in a custom non-magnetic loading device designed for use in a 9.4T Bruker Avance 400 [3]. For DENSE-FSE, IVDs were preconditioned with 200 30-N intermittent compressive cycles at 0.33Hz, and displacement-encoded data in the image slice was acquired as axial compression continued for 576 more cycles (TR=3s; TE=24.7ms; interlace=8; FOV=16mm^2, matrix=128x128; slice thickness=1mm; averages=4). For dGEMRIC, a variable repetition time mutli-slice multi-excitation (MSME) sequence (TE=7.2ms; FOV=16mm^2; matrix=256x256; slice thickness=1mm) was used to image a coronal image slice before and after equilibrium in 2mM Gd-DTPA^{2-} in PBS. Paravision (4.0, Bruker, Ettlingen, Germany) was used to define spline-fit regions of interest in MR images prior to further image processing. GAG content was computed for the whole disc [4] using Matlab (R2008a, Mathworks, Natick, MA). After unwrapping phase information from DENSE-FSE data, displacements in the loading and transverse directions of each pixel within the whole disc were also computed using Matlab, and displacement fields were then smoothed [3]. Immediately after imaging, specimens were prepared for histology and stained with hematoxylin and eosin (H&E). Histological slides were digitally photographed in sections and then composited using Photoshop (CS4, Adobe, San Jose, CA).

RESULTS: Whole disc GAG content (n = 8) averaged 307.9±109.8 μg/mg in L3/L4 IVDs and 162.2±27.0 μg/mg in L4/L5 IVDs, which were punctured with a needle. A histological slice through the disc was taken for a representative specimen set (Figure 1), whose GAG content as a pair was closest to the average. For this specimen, smoothed displacements in the loading and transverse directions ranged from -0.03 to 0.19 mm and from -0.18 to 0.22 mm, respectively, for the L3/L4 IVD and from -0.08 to 0.17 mm and from -0.09 to 0.35 mm, respectively, for the punctured L4/L5 IVD.

DISCUSSION: In this study, DENSE-FSE, a noninvasive MRI method to quantify the mechanical behavior of tissues, was used to evaluate the degeneration of intervertebral discs in a rabbit model. In addition, dGEMRIC, a MRI technique to measure GAG content, and histological staining was used to confirm the results of the degeneration model. A previous dGEMRIC study found that GAG concentration decreased with more advanced stages of degeneration [7], a decrease observed in this study. Researchers have also previously correlated the GAG content of the nucleus pulposus with the mechanical behavior of IVDs under axial loading [8]. Previous studies that determine the displacement of IVDs examine bulk mechanical behavior [8], use embedded pressure transducers [9], and utilize texture correlation and interpolation [10]. On the other hand, DENSE-FSE enables displacements to be measured noninvasively for every resolved pixel at absolute precisions exceeding 8.8 μm [3], allowing for a direct comparison of the mechanical behavior to the GAG concentration. This is especially important in the evaluation of degenerated IVDs, where the nucleus pulpopsus is damaged, as is the case for a puncture model of degeneration. From displacement fields, it is possible to then compute the strains [11] and even stresses and material properties using appropriate (e.g. non-linear) constitutive models.

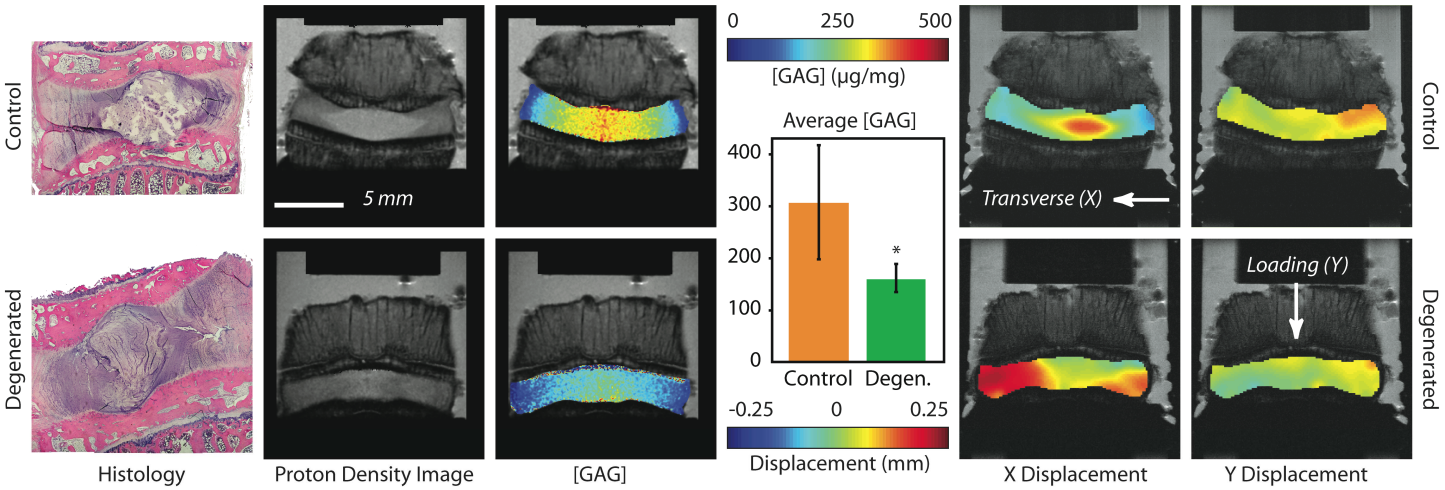

Figure 1: The control (L3/L4) and degenerated (L4/L5) discs are compared using histology, dGEMRIC, and DENSE-FSE. Histology shows an intact nucleus pulposus surrounded by the annulus fibrosis in the control disc, whereas the degenerated disc is fibrillated throughout. Proton density MR images are shown for both discs and the map of GAG concentration is overlaid on the same magnitude image. Average [GAG] pooled over all pairs of IVDs (n = 8) show a significant difference between [GAG] in control versus degenerated discs (p < 0.01; paired t-test) after 28 days. Smoothed displacement fields are overlaid on a MR image taken during the loading plateau of cyclic compression. Arrows indicate the coordinate system in the loading (+Y) and transverse (+X) directions.

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