Mechanical Deformation and Glycosaminoglycan Content Changes in a Rabbit Annular Puncture Disc Degeneration Model

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Study Design. Evaluation of degenerated intervertebral discs from a rabbit annular puncture model by using specialized magnetic resonance imaging (MRI) techniques, including displacement encoding with stimulated echoes and a fast-spin echo (DENSE-FSE) acquisition and delayed gadolinium-enhanced MRI of cartilage (dGEMRIC).

Objective. To evaluate a rabbit disc degeneration model by using various MRI techniques. To determine the displacements and strains, spin-lattice relaxation time ($T_1$), and glycosaminoglycan (GAG) distribution of degenerated discs as compared to normal and adjacent level discs.

Summary of Background Data. Annular puncture of the intervertebral disc produces disc degeneration in rabbits. DENSE-FSE has been previously demonstrated in articular cartilage for the measurement of soft tissue displacements and strains. MRI also can measure the $T_1$ of tissue, and dGEMRIC can quantify GAG concentration in cartilage.

Methods. In eight New Zealand white rabbits, the annulus fibrosis of a lumbar disc was punctured. After 4 weeks, the punctured and cranially adjacent motion segments were isolated for MRI and histology. MRI was used to estimate the disc volume and map $T_1$. DENSE-FSE was used to determine displacements for the estimation of strains. dGEMRIC was then used to determine GAG distributions.

Results. Histology and standard MRI indicated degeneration in punctured discs. Disc volume increased significantly at 4 weeks after the puncture. Displacement of the nucleus pulposus was distinct from that of the annulus fibrosis in most untreated discs but not in punctured discs. $T_1$ was significantly higher and GAG concentration significantly lower in punctured discs compared with untreated adjacent level discs.

Conclusion. Noninvasive and quantitative MRI techniques can be used to evaluate the mechanical and biochemical changes that occur with animal models of disc degeneration. DENSE-FSE, dGEMRIC, and similar techniques have potential for evaluating the progression of disc degeneration and the efficacy of treatments.

Key words: rabbit disc degeneration, displacement-encoded MRI, dGEMRIC, cartilage elastography, quantitative MRI. Spine 2011;36:1438–1445

Low back pain, which affects more than 25 million people in the United States, has serious personal and societal impact. 1 Although the etiology of this pain remains largely unknown, there is a clinical link between degenerate intervertebral discs and chronic low back pain. 2 Disc degeneration is an age-related process that involves both biochemical and mechanical changes. 3

Animal models of intervertebral disc degeneration can enhance understanding of the progression of disc degeneration and provide a means to evaluate treatment regimens. 4 The rabbit annular puncture model 5,6 can be evaluated by using various techniques. Histology allows for the localization of microscale disc structure. Mechanical testing of the disc throughout the degeneration process in an animal model provides valuable information about the functional integrity of the disc. Despite the utility of these techniques, sacrifice of the animal is required, and this limits the number of specimens that can be examined at each time point in longitudinal studies. Instead, noninvasive imaging techniques can be used to evaluate the changes that occur in longitudinal studies of animal models.

Noninvasive medical imaging can be used to monitor changes in animal models of disc degeneration. Although previous studies of the rabbit puncture model used radiography to evaluate changes in disc height index 7 and the morphology of vertebral body bone, 8 measuring disc height from projection images can result in variations in disc height indexes. 9 Magnetic resonance imaging (MRI), a technique more appropriate than radiography for directly visualizing soft tissues, can be

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used to grade the extent of degeneration in animal models\textsuperscript{5,8} and to quantify changes in the area and signal intensity of the nucleus pulposus.\textsuperscript{9} Studies in humans, however, have shown that disc height and signal intensity may not be sensitive to the early changes that occur in disc degeneration.\textsuperscript{9} Although MRI is well suited to provide morphologic and structural information, some quantitative MRI techniques have shown promise for detecting changes that occur in early disc degeneration.\textsuperscript{10,11}

Quantitative MRI is used to characterize the mechanical and biochemical characteristics of soft tissue, including measures of the displacement and strain patterns in soft tissues and glycosaminoglycan (GAG) content. Displacement encoding with stimulated echoes and a fast-spin echo (DENSE-FSE) readout is a phase-contrast MRI technique that allows for the direct measurement of displacements that occur during cyclic loading. DENSE-FSE has been used in articular cartilage explants\textsuperscript{12} and intact tibiofemoral joints.\textsuperscript{13} Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is a quantitative MRI technique that determines the fixed charge density and GAG distribution within tissue by using the contrast agent gadopentetate dimeglumine (Gd-DTPA\textsuperscript{2−}).\textsuperscript{14} Gadolinium-enhanced imaging has previously been used to improve evaluation of intervertebral discs\textsuperscript{15} and to quantify GAG in the nucleus pulposus.\textsuperscript{16} Rabbit model disc biochemistry has been quantified with high-field MRI,\textsuperscript{17} but dGEMRIC has not been used to evaluate GAG content in an animal model of disc degeneration. Finally, despite the importance of biomechanics in disc function, displacement-encoded MRI and elastography have not yet been applied to either healthy or degenerated intervertebral discs.

To ultimately address repair and regeneration of intervertebral disc degeneration in longitudinal studies, the purpose of this article is to evaluate a rabbit annular puncture disc degeneration model by using quantitative MRI. The objectives were threefold, namely, to (1) evaluate the morphologic changes in the annular puncture model of disc degeneration by histology and MRI, (2) determine the displacement and strain patterns by DENSE-FSE, and (3) quantify changes in GAG concentration by dGEMRIC.

MATERIALS AND METHODS

Degeneration Model

Eight skeletally mature, male New Zealand white rabbits (weight ∼3.5 kg) were treated in an annular puncture degeneration model,\textsuperscript{1} while three were used as controls, under Institutional Animal Care and Use Committee approval. For each treated rabbit, the intervertebral disc between the fourth and fifth vertebrae (L4–L5) was punctured by using a 16-gauge needle under aseptic conditions. Lateral radiographs of the lumbar spine were taken during surgery and before sacrifice. Rabbits were killed 4 weeks postoperation, and the lumbar spines, which include the cranial and untreated disc (L3–L4) and L4–L5 discs, were isolated. Lumbar spines were also isolated from the control animals.

L3–L4 and L4–L5 motion segments were trimmed to include the disc and approximately 5 mm of the cranial and caudal vertebral bodies. Specimens were mounted in an MRI-compatible, cyclic loading device with cyanoacrylate and bathed in phosphate-buffered saline (PBS).\textsuperscript{12}

Magnetic Resonance Imaging

All motion segments were imaged by using a vertical 400 MHz MRI system (9.4 Tesla, Bruker Medical GMBH, Ettlingen, Germany). Fast-spin echo (FSE) scans were acquired in adjacent coronal slices through the disc by using the following parameters: repetition time (TR) = 3000 milliseconds, echo time (TE) = 5.3 milliseconds, field of view (FOV) = $25.6 \times 25.6$ mm\textsuperscript{2}, matrix size = $256 \times 256$ pixels, slice thickness = 1.0 mm, interslice distance = 1.0 mm, and number of slices = 9. The adjacent 1-mm slices were used to estimate the intervertebral disc volume with ImageJ (National Institutes of Health, Bethesda, MA). Regions of interest (ROIs) containing the intervertebral disc were manually segmented in all image slices that passed through the disc. The ROI areas were then used to estimate disc volume. The initial FSE scans were additionally used to select a coronal slice across the largest width of the disc for subsequent imaging. After slice selection, specimens were not repositioned or removed until the completion of all imaging.

Displacement Encoding with Stimulated Echoes and Fast-Spin Echo

Displacement and strain patterns were determined by DENSE-FSE. A computer-controlled electropneumatic system was used to synchronize cyclic compression and DENSE-FSE imaging.\textsuperscript{12} Every 3.0 seconds, 30 N of compression was applied for 1.5 seconds, with MRI readout during this loading plateau. To prevent blurring of images due to changes in the deformation of the disc between cycles, DENSE-FSE images were not acquired until after 200 cycles of compression to ensure a steady-state load-displacement response.\textsuperscript{12} A displacement-encoding gradient was applied before and during loading. For calculation of displacements, DENSE-FSE requires phase information from a reference scan, during which the displacement-encoding gradient off, and scans with displacement-encoding gradients in orthogonal directions (i.e., x, y).\textsuperscript{12} Imaging parameters were as follows: effective TR = 3000 milliseconds; effective TE = 24.7 milliseconds; interleave factor = 8; FOV = $16 \times 16$ mm\textsuperscript{2}; matrix size = $128 \times 128$ pixels; slice thickness = 1.0 mm; and number of averages = 4. Displacement-encoding gradients in the loading and transverse directions were 95 mT/m, with an effective duration of 1.0 milliseconds. Using a standard FSE sequence, images of the deformed specimen were also acquired, and the deformed disc was manually segmented by using Paravision (Bruker Medical GMBH, Ettlingen, Germany).

Image processing software (Matlab v7.0, The Mathworks, Natick, MA) was used to determine displacements and estimate Green-Lagrange strains from smoothed displacement fields.\textsuperscript{12} For comparisons of trends between discs, displacements and strains along vertical lines of interest, which were chosen along the geometric center of the full ROI, were normalized along the disc depth.
Delayed Gadolinium-Enhanced Magnetic Resonance Imaging of Cartilage

GAG patterns were determined by dGEMRIC, using MR acquisitions before and after the administration of an anionic contrast agent. A variable TR multislice multiecho (MSME) scan was acquired through the selected slice with the following parameters: TRs = 100, 200, 300, 500, 900, and 4000 milliseconds; TE = 7.2 milliseconds; FOV = 16 × 16 mm²; matrix size = 256 × 256 pixels; and slice thickness = 1.0 mm. The PBS bath was then replaced with PBS containing 2 mmol/L Gd-DTPA²⁻. Sufficient time (>9 hours) was allowed for the gadolinium to penetrate into the immersed disc. A set of MSME scans was then acquired under the same imaging parameters, except that TRs = 100, 200, 300, 500, 900, and 2500 milliseconds.

MR imaging software (Paravision 3.0, Bruker Medical GMBH, Ettlingen, Germany) was used to calculate the spin-lattice relaxation time constants (T₁) within the imaged slice before and after the administration of Gd-DTPA²⁻. An ROI in the MSME image of the undeformed disc was then defined manually by using closed Bezier curves. Computational software (Matlab) was then used to calculate fixed charge density and GAG concentration. T₁, before and after Gd-DTPA²⁻ administration, and GAG concentration, were averaged across the disc.

Histology

All motion segments were fixed in a 10% formaldehyde solution immediately after the completion of all MRI tests. Fixed specimens were decalcified by using 10% formic acid and embedded in paraffin before slicing. Histologic slices were then stained with hematoxylin and eosin and visualized by light microscopy.

Statistics

T₁ values and GAG content between control L3–L4 and control L4–L5 and between untreated L3–L4 and punctured L4–L5 were compared by using a paired Student t test. P < 0.05 indicated significance. All values were reported as mean ± standard error of the mean.

RESULTS

Degeneration Model Morphology

Morphologic changes were observed in degenerated discs (Figure 1). Lateral radiographs (Figure 1A) confirmed that the discs between the fourth and fifth lumbar vertebrae were treated with needle puncture of the annulus fibrosis. Hematoxylin and eosin staining showed intact nucleus pulposus and annulus fibrosis in untreated L3–L4 and punctured L4–L5 were compared by using a paired Student t test. P < 0.05 indicated significance. All values were reported as mean ± standard error of the mean.

In contrast, the volume of untreated L3–L4 discs (1.148 ± 0.054 cm³) was significantly less (P = 0.02) than the volume of punctured L4–L5 discs (1.283 ± 0.095 cm³).

Figure 1. The morphology of the intervertebral disc is altered in the annular puncture disc degeneration model. Lateral radiographs (A), shown for a representative rabbit, were used to confirm the anatomic location of the untreated and punctured discs. Histologic slices were then taken through the center of the disc in approximately the same location as the volumetric slice for MRI (B). Hematoxylin and eosin histology (C) and standard MR images (D) are shown for the untreated and punctured discs of a representative treatment rabbit.
Displacement and Strain Patterns
Altered displacement patterns were observed in punctured discs compared with controls (Figure 2). While no significant differences were observed between untreated L3–L4 and punctured L4–L5 groups when displacements were averaged over all specimens in the loading (P = 0.60) and transverse directions (P = 0.55), displacement patterns varied for each specimen depending on the treatment (Figure 2B). For most control and untreated L3–L4 specimens, but not punctured L4–L5 specimens, displacement patterns showed that, under cyclic compression, the nucleus pulposus displaced transversely with respect to the surrounding annulus fibrosis.

Strains, as computed from smoothed displacement fields, were also heterogeneous throughout the discs (Figure 3). However, the averaged strain values within untreated L3–L4 and treated L4–L5 discs were not significantly different (axial, P = 0.41; transverse, P = 0.59; shear, P = 0.40). Mostly tensile and some compressive strains were observed in the direction transverse to loading, but predominantly, compressive strains were seen in the loading direction. Strains (and displacements), normalized through the depth of untreated and punctured discs, showed large variations (Figure 4).

T₁ Relaxation and GAG Concentration
T₁ maps indicated changes in distribution with degeneration (Figure 5A), but T₁ averaged across the full disc showed no significant differences between untreated L3–L4 and punctured L4–L5 groups (P = 0.20; Table 1). GAG concentration in punctured L4–L5 discs was significantly less than that in the untreated L3–L4 discs (P = 0.015; Table 1). In addition, the GAG distributions of untreated L3–L4 discs differed from those of punctured L4–L5 discs, with most of the difference in GAG concentration observed in the nucleus pulposus (Figure 5B).

Table 1. Changes in Disc Volume, Spin-Lattice Relaxation Time (T₁), and GAG Content After Disc Degeneration

<table>
<thead>
<tr>
<th></th>
<th>Estimated Whole Disc Volume (mm²)</th>
<th>Spin-Lattice Relaxation Time (ms)</th>
<th>Glycosaminoglycan Content (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control L3–L4 (mean ± SEM)</td>
<td>898 ± 83</td>
<td>1043 ± 270</td>
<td>388 ± 111</td>
</tr>
<tr>
<td>Control L4–L5 (mean ± SEM)</td>
<td>988 ± 74</td>
<td>1221 ± 74</td>
<td>252 ± 24</td>
</tr>
<tr>
<td>Paired t test (n = 3)</td>
<td>P = 0.51</td>
<td>P = 0.55</td>
<td>P = 0.26</td>
</tr>
<tr>
<td>Untreated L3–L4 (Mean ± SEM)</td>
<td>1148 ± 54</td>
<td>1213 ± 108</td>
<td>301 ± 41</td>
</tr>
<tr>
<td>Punctured L4–L5 (Mean ± SEM)</td>
<td>1283 ± 95</td>
<td>1397 ± 31</td>
<td>162 ± 10</td>
</tr>
<tr>
<td>Paired t test (n = 8)</td>
<td>P = 0.02</td>
<td>P = 0.20</td>
<td>P = 0.015</td>
</tr>
</tbody>
</table>

Whole-disc volume was estimated by using MRI from adjacent image slices through the full intervertebral disc. T₁ was measured by using standard MRI relaxometry techniques, and glycosaminoglycan content was determined by using dGEMRIC. There was a significant difference in volume and glycosaminoglycan content between the untreated L3–L4 and punctured L4–L5 groups.
DISCUSSION
This study characterized mechanical and biochemical changes in a rabbit annular puncture model. Disc degeneration was confirmed by changes in morphology observed in histology and with standard MRI. Displacement-encoded MRI was used to demonstrate differences in mechanical behavior between intact and punctured discs. Although average displacement and strain values showed no significant difference between untreated and punctured discs across multiple animals, displacement patterns were altered with annular puncture within each animal. MRI showed a slight decrease in $T_1$, and Gd-DTPA$^{2-}$ contrast was used to quantify the significant decrease in GAG content with degeneration.

Disc morphology and volume are altered in degenerated discs. Histologic staining showed that fibrillation of the nucleus pulposus occurred in punctured discs, which is in agreement with previous studies of the rabbit annular puncture model. In this study, punctured L4/L5 discs had a significantly higher disc volume than untreated L3/L4 discs after annular puncture (Table 1). Although unexpectedly there were no significant differences between the control L3/L4 and L4/L5 discs, natural differences in size between L3/L4 discs and L4/L5 discs, which are more caudal, could contribute to the measured difference in the punctured discs. The puncture degeneration model, especially with larger-diameter needles, results in ruptured fibers in the annulus, which can affect its ability to resist internal swelling pressure. Therefore, the increase in disc volume could result from the reduced ability of the annulus to resist disc pressure. In addition, although not statistically significant, the slight increase in $T_1$ may also indicate that more free water is present in the punctured discs (Table 1, Figure 5A). Previous studies show either no change or a decrease in $T_1$ with advanced disc degeneration. However, degeneration progressed slowly with this model, and animals were killed after only 4 weeks, so tissue swelling might occur early in the degenerative process.

Along with changes in disc morphology and biochemical components, altered mechanical behavior is seen with aging and degenerated discs. Changes to the pressure profile within the nucleus pulposus have previously been measured, and loading patterns in the periphery of the disc are also altered with degeneration. The puncture model of disc degeneration results in decreased compressive stiffness of the disc,

Figure 3. Strain patterns in the transverse and loading directions and in shear vary within representative control (A) and treatment (B) animals. Strains patterns are derived from smoothed displacement fields and, as such, appear similar between untreated and punctured discs.
allows for further degeneration of the disc.\textsuperscript{19,24} In this study, displacements of the nucleus pulposus in most control and untreated discs, but not treatment discs, were distinct from those of the annulus fibrosis. Previous studies have reported that the nucleus pulposus translates in response to bending loads in the direction opposite to bending.\textsuperscript{25} Because transverse displacement of the nucleus pulposus was observed, it is likely that, although only compressive loads were applied to the motion segment, the disc itself experienced bending because of the geometry of discs in relation to the loading axis. Although every effort was made to make the parallel cuts of the vertebral bodies orthogonal to the middle plane of the intervertebral disc, an approximation of the angle of the discs showed that the discs in this study were $-0.22^\circ \pm 5.40^\circ$ off from perpendicular to the loading axis. Because of the range in both the displacement and strain values (Figure 4), comparisons between discs

Figure 4. Displacements and strains were normalized through depth and averaged across all discs. Averaged displacements show similar trends in untreated and punctured discs. Green-Lagrange strains are computed from smoothed displacements and then normalized and averaged. Standard error bars indicate variability in displacement and strain values within the same treatment group.

Figure 5. Spin-lattice relaxation time ($T_1$) and glycosaminoglycan (GAG) distribution, as determined by using dGEMRIC, altered with annular puncture. $T_1$ maps (A) and GAG content (B) in the unloaded state are shown for a representative control and treatment animal. $T_1$ decreases and GAG is lost with degeneration in punctured L4–L5 discs.

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in the same animal (Figures 2 and 3) may be more useful than generalizations based on a group. Smoothing of displacement data to estimate strain fields can also lead to the loss of detail. In addition, the displacement and strain patterns should be examined with consideration of the unique loading environment. It is also pertinent to note that, because discs are affected by an adjacent degenerated disc,26 the untreated L3–L4 disc may not be representative of completely healthy discs.

In this study, GAG concentration in the punctured discs was significantly decreased from that of untreated discs (Table 1). GAG content increases from the annulus toward the nucleus in normal discs.27 Herein, GAG content in the nucleus decreased with degeneration, but degenerated discs showed minimal change in GAG in the annulus fibrosis (Figure 5). Proteoglycan loss in early disc degeneration has previously been shown to occur predominantly in the nucleus pulposus rather than the annulus fibrosis.1,2,11 Loss of proteoglycans in the nucleus with degeneration29,30 explains the flattening of the profile of GAG throughout the width of the disc that has been reported in previous studies.2 The loss of GAG content can also be in part due to herniation of the nucleus pulposus during or immediately after surgery.

This study uses quantitative MRI for the evaluation of the biochemical and mechanical outcome of a rabbit puncture model of disc degeneration. Because MRI is noninvasive, these methods are translatable to in vivo use for longitudinal studies of disc degeneration models and various interventions. In order for the transition to in vivo use to occur, however, several limitations of these techniques must be considered, as outlined subsequently.

Displacement-encoded MRI allows for the characterization of displacements and strains in the degenerated disc, but the DENSE-FSE technique retains several technical limitations. Because the precision of strains is limited by the signal-to-noise ratio of the phase data,12 higher signal-to-noise ratio would permit less destructive filtering of displacement fields and prevent loss of detail. In addition, long imaging times make the current DENSE-FSE technique impractical for in vivo use. Nevertheless, using MRI to determine mechanical behavior of degenerated discs retains an advantage due to its noninvasive nature. Current methods require invasive procedures to access the disc for measurement of mechanical properties,13 are feasible only in an ex vivo environment, or disrupt the loading environment of the disc.25 MRI has previously been used to measure nominal displacements and strains in discs under compression26 and torsion,11 correlate GAG content to disc mechanics,14 and determine strains using texture correlation.33 Displacement-encoded MRI, however, provides displacement data for every pixel of interest, with no bias in computed displacements and strains and no interpolation.12 Data from such a technique allows for the evaluation of the spatial dependence of mechanical behavior in tissues with heterogeneous material properties. Future work in displacement-encoded MRI should aim to produce even faster imaging times, higher signal-to-noise ratio, and improved denoising techniques during image processing.

The use of quantitative MRI techniques in correlation with biochemical components also has several limitations that must be considered. Use of gadolinium contrast to quantify GAG content requires that Gd-DTPA be able to penetrate evenly into the tissues of interest.26 For administration in vivo, the route of penetration of contrast agent into the disc is through the vertebral body endplate.27 However, with age and degeneration, diffusion through the endplate into the intervertebral disc may be affected.17,18 This limits the penetration of gadolinium into the disc18 and, therefore, the interpretation of in vivo dGEMRIC data.36 However, in this study, the discs were submerged in the Gd-DTPA solution and were allowed adequate time (9 hours) for contrast agent penetration before imaging. Although studies have shown that T1 after gadolinium administration can correlate with degeneration,16 the design of future studies must also consider other quantitative MRI techniques that have shown promise for imaging disc degeneration. Spin–spin relaxation time (T2) can be correlated to water content in the disc,40 to collagen content,20 and, in highly degenerated discs, to proteoglycan content.41 In addition, studies of spin-lattice relaxation time in the rotating frame (T1ρ) in degenerated discs have shown good correlation with proteoglycan content,42 GAG content,10 and also water content and swelling pressure.43 Nonetheless, the mechanism of correlation between contrast agent concentration and GAG content has been well established for dGEMRIC.14,36

In summary, this study evaluated morphologic, biochemical, and mechanical changes in a rabbit annular puncture disc degeneration model by using histology, standard MRI, dGEMRIC, and DENSE-FSE. Overall GAG content decreased in treatment discs, with loss of localization of GAG in the nucleus pulposus. Displacement of the nucleus pulposus was distinct in control and untreated discs but not in treatment discs. In addition, comparisons of mechanical behavior should be performed on a per-animal basis because variability in displacements and strains between animals may be on the same order as changes within a single animal. Despite current limitations, a combination of quantitative MRI techniques like dGEMRIC and DENSE-FSE can provide valuable information about changes that occur in early stages and throughout the progression of disc degeneration.

Key Points

- Biochemical and mechanical changes with annular puncture of rabbit lumbar intervertebral discs were evaluated by using DENSE-FSE and dGEMRIC.
- Morphologic changes in the disc degeneration model were confirmed by using histology and MRI.
- Displacements determined by using DENSE-FSE and calculated strains demonstrated that the motion of the nucleus pulposus was distinct from that of the annulus fibrosis for untreated but not for punctured discs.
- Imaging with dGEMRIC showed a decreased localization of GAGs in the nucleus pulposus and an overall decrease in average GAG content in punctured discs compared with untreated discs.
References


