Recapitulating the Spectrum of Intervertebral Disc Degeneration in a Large Animal Model

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Introduction

Intervertebral disc degeneration is a progressive cascade that leads to structural and mechanical failure, and is frequently associated with low back pain. Patients present in a variety of degenerative states, classically defined by the Pfirrmann grading system, based on qualitative interpretation of magnetic resonance images (MRI).[1] Current treatments focused on alleviating the symptoms of discogenic pain are limited, as they do not restore disc mechanics or structure. As a result, there is considerable interest in developing biologic regenerative therapies to treat disc degeneration. For these therapies to be successful they must be tailored to the degenerative state of each disc. For example, they may include injection of stem cells, hydrogels or growth factors for mild to moderately degenerated discs, or engineered total disc replacements for severely degenerated discs.[2,3] A critical aspect of evaluating and translating these regenerative approaches into clinical use is a size-relevant large animal model that recapitulates the spectrum of degeneration seen in humans. The objective of this study was to establish a goat model of intervertebral disc degeneration in which a gradient of degenerative changes, from mild to severe, could be reproducibly achieved through mechanical (nucleotomy) or chemical (chemonucleolysis) perturbation.

Materials & Methods

With IACUC approval, 9 goats underwent a surgical procedure to induce the degeneration of the lumbar intervertebral discs. Using an open, lateral, retroperitoneal transpsoatic approach, L1-2, L2-3 and L3-4 lumbar discs were randomized to receive either subtotal nucleotomy (n = 10) or injection of 200μL of either 0.1U (n = 5), 1U (n = 10) or 5U (n = 5) chondroitinase-ABC (ChABC) via a 22G spinal needle. The L4-L5 (n = 5) disc received a sham saline injection, and the T13-L1 and L5-L6 discs served as intact controls (n = 10). Lateral plain radiographs of the lumbar spine were obtained pre-operatively, immediately post-operatively, and at 1, 2, 4, 6, 8, 10 and 12 weeks post-operatively for quantification of disc height index (DHI). After 12 weeks, the animals were euthanized, and the lumbar spines harvested and imaged using a 3T MRI scanner. Images for quantitative T1δ and T2 mapping[4] were obtained, as well as T2 weighted images for Pfirrmann grading. Correlations between quantitative MRI parameters and Pfirrmann grade were established by linear regression.

Individual motion segments were then imaged using high resolution microcomputed tomography (μCT) to visualize and quantify morphologic changes to the vertebral bony endplate. Alcian blue (glycosaminoglycans) and picrosirius red (collagen) stained, mid-sagittal, histological sections were used to visualize degenerative changes. Sections were graded on a visual analog scale by three blinded observers in five categories: organization of the annulus fibrosus, nucleus pulposus matrix, annulus fibrosus/nucleus pulposus border, nucleus pulposus cellularity, and cartilage endplate structure. Five samples from each of the intact control, nucleotomy and 1U ChABC groups were utilized for biomechanical testing. Following overnight equilibration in PBS, samples were subjected to a testing protocol consisting of 20 cycles tension compression (−230N to +115N), followed by 1 hour of creep at −230N (~0.48 MPa).[5] Tension/compression data was fit to a sigmoid function and creep data was fit to a five parameter viscoelastic constitutive model for analysis.[6] For quantitative outcome measures, statistically significant differences ($p < 0.05$) were established via one or two-way ANOVAs with Tukey's post-hoc tests.

Results

Histological evaluation (Figure 1) revealed advanced degenerative changes to the disc in the 5U ChABC group, with moderate changes in the nucleotomy and 1U groups, and mild changes to the 0.1U ChABC group. Histologic scores were highest in the 5U and 1U ChABC group, followed by the nucleotomy and 0.1U ChABC groups across all categories. There were no statistically significant differences in histologic score between control and sham discs. Discs injected with 1U or 5U of ChABC exhibited a progressive decrease in DHI, with 28% and 34% reductions in DHI in the 1U and 5U groups, respectively, after 12 weeks ($p < 0.05$). Compared to the sham group, DHI was also significantly reduced in the 5U group at 10 weeks, and in the 1U group at 2, 4, 6, 8, and 10 weeks ($p < 0.05$). Discs subjected

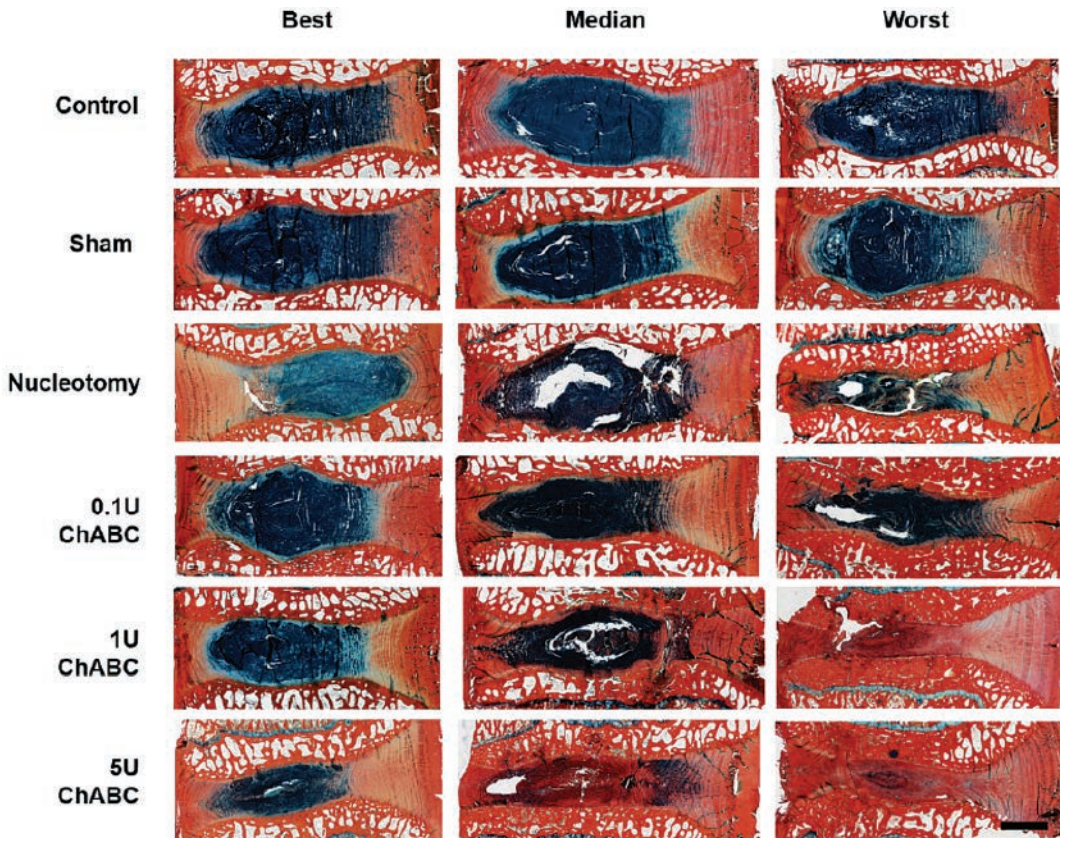

Figure 1. Histology. Degeneration was characterized by fibrosis of the nucleus pulposus, disorganization of the annulus fibrosus, loss of disc height and reduction in alcian blue staining. No degenerative changes were observed in sham discs compared to control. Alcian blue and picrosirius red stain, scale bar = 3 mm.

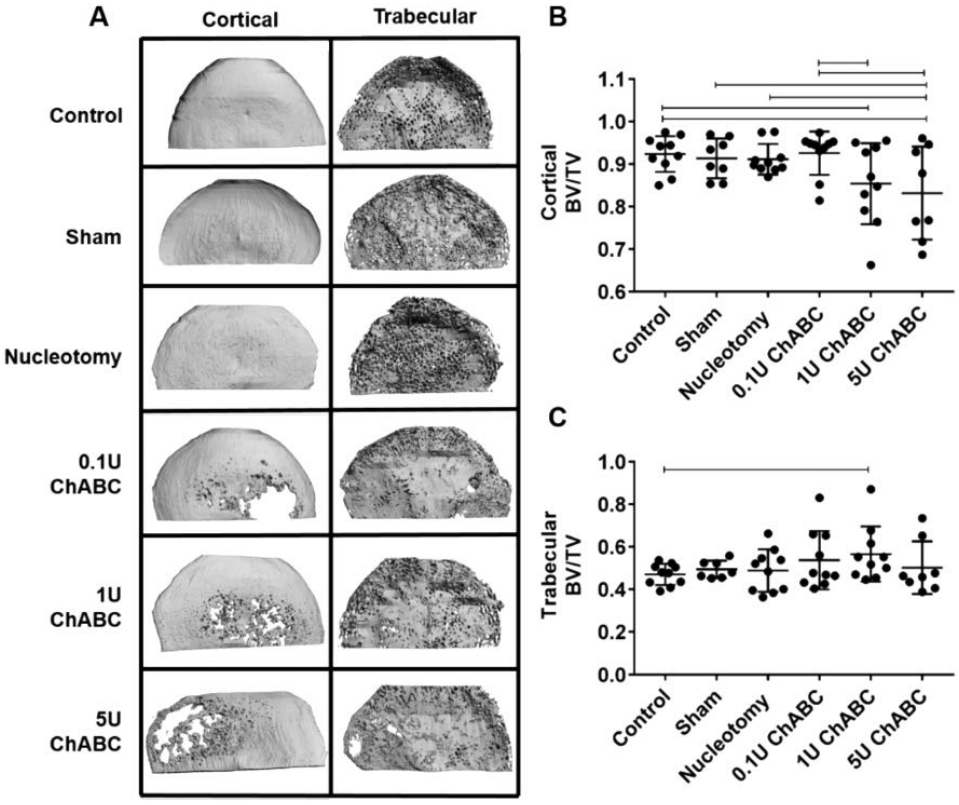

Figure 2. Microcomputed tomography (μCT). 3D reconstructions (A) of the subchondral cortical endplate and the adjacent trabecular bone illustrate alterations to the cortical (B) and trabecular (C) bone volume fraction. The cortical endplate with the lowest BV/TV is shown in (A). Bars denote significance, $p < 0.05$, ANOVA, Tukey's post-hoc test.

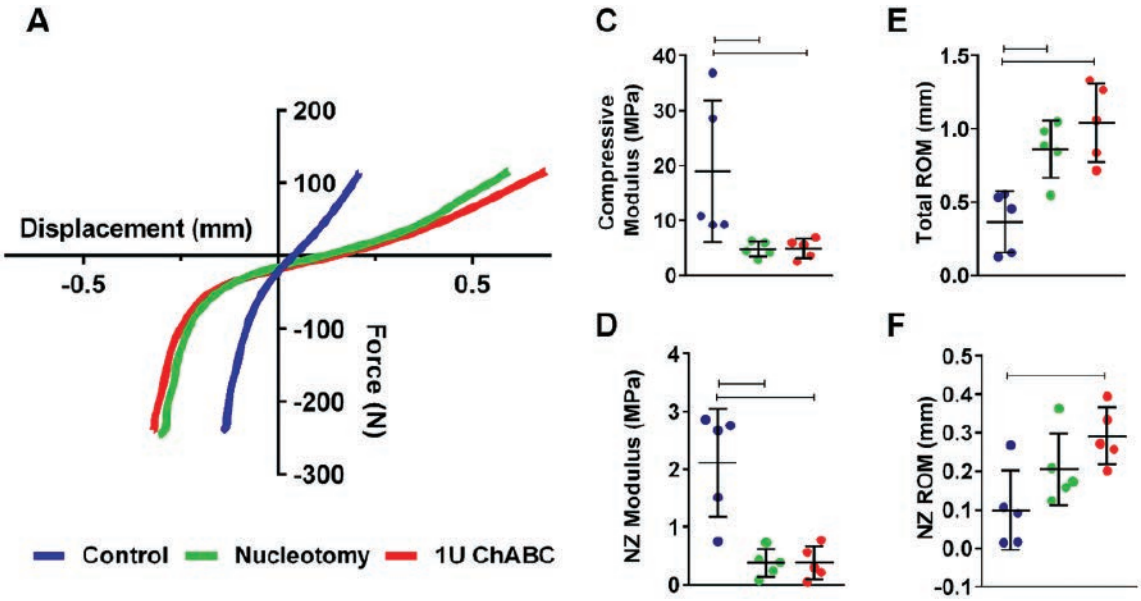

Figure 3. Biomechanical properties. Average force displacement curves (A) generated by LOWESS smoothing demonstrate aberrations in disc mechanical properties with degeneration. Significant differences in compressive modulus (C), NZ modulus (D), Total ROM (E) NZ ROM (F) were observed in degenerative versus control discs. Bars denote significance, $p < 0.05$, ANOVA, Tukey's post-hoc test.

to nucleotomy or injection of 0.1U ChABC also exhibited an initial decrease in DHI (significant at 1, 4, 6, 8 and 10 weeks); however, partial recovery of disc height was observed at 12 weeks in both groups. DHI for control and sham injected discs was unchanged over 12 weeks.

Three-dimensional μCT analysis (Figure 2) demonstrated significant cortical bone loss in the vertebral endplate of the 1U and 5U groups. The bone volume fraction of the adjacent trabecular bone was significantly increased for these same groups. T1δandT2 values in the nucleus pulposus (NP) were significantly lower in the 1U and 5U groups compared to sham and control discs. Nucleus pulposus T2 values were significantly lower in the nucleotomy group compared to sham.T1δandT2 values correlated significantly ($r^2 = 0.63$ and $r^2 = 0.53$, respectively) with Pfirrmann grades. Average force displacement curves (Figure 3) illustrated alterations to the mechanical response of the disc to loading in the 1U ChABC and nucleotomy groups compared to control. Compressive and neutral zone modulus were significantly lower, and range of motion greater, in nucleotomy and 1U ChABC discs compared to controls.

Discussion

A large animal goat model of disc degeneration was established that exhibits a gradient of degenerative changes from mild to severe. This work advances previous caprine animal models of degeneration, which have mainly achieved only mild degenerative changes.[7] Observed changes to both the disc and the bone in the adjacent vertebral endplate are consistent with the changes reported in human disc degeneration.[8,9] Over the 12 week study, nucleotomy and injection of low dose (0.1U) ChABC induced mild to moderate degenerative changes to the disc, as seen via disc height changes, quantitative MRI, and histology. Injection of higher doses (1U and 5U) ChABC induced moderate to severe disc degeneration. Structural derangement of the disc with degeneration in the 1U ChABC and nucleotomy groups was associated with significantly altered disc mechanical function.

Conclusions

In this study, we established a large animal model that replicates the spectrum of disc degeneration seen in humans and provides the basis for future studies of the biological mechanisms underlying disease progression. Moreover, this model can be used to evaluate the therapeutic potential and safety of a wide range of novel biological therapies designed to treat disc degeneration at a variety of stages.

References

1. Pfirrmann CW, Metzdorf A, Zanetti M, *et al.* 2001. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine* (Phila. Pa. 1976). 26:1873–8.

2. Sakai D, Andersson GBJ. 2015. Stem cell therapy for intervertebral disc regeneration: obstacles and solutions. *Nat. Rev. Rheumatol.* 11:1–14.

3. Mizuno H, Roy AK, Zaporojan V, *et al.* 2006. Biomechanical and biochemical characterization of composite tissue-engineered intervertebral discs. [Internet].*Biomaterials* 27(3):362–70.

4. Martin JT, Collins CM, Ikuta K, *et al.* 2015. Population average T2 MRI maps reveal quantitative regional transformations in the degenerating rabbit intervertebral disc that vary by lumbar level. *J. Orthop. Res.* 33:140–148.

5. Beckstein JC, Sen S, Schaer TP, *et al.* 2008. Comparison of animal discs used in disc research to human lumbar disc: axial compression mechanics and glycosaminoglycan content. *Spine* (Phila. Pa. 1976). 33:E166–73.

6. Martin JT, Gorth DJ, Beattie EE, *et al.* 2013. Needle puncture injury causes acute and long-term mechanical deficiency in a mouse model of intervertebral disc degeneration.*J. Orthop. Res.* 31:1276–1282.

7. Hoogendoorn RJW, Helder MN, Kroeze RJ, *et al.* 2008. Reproducible long-term disc degeneration in a large animal model. [Internet].*Spine* (Phila. Pa. 1976). 33:949–54.

8. Haefeli M, Kalberer F, Saegesser D, *et al.* 2006. The course of macroscopic degeneration in the human lumbar intervertebral disc.*Spine* (Phila. Pa. 1976). 31:1522–1531.

9. Rutges JPHJ, Jagt van der OP, Oner FC, *et al.* 2011. Micro-CT quantification of subchondral endplate changes in intervertebral disc degeneration [Internet].*Osteoarthr. Cartil.* 19(1):89–95.