A Large Animal Model of Motion Segment Degeneration for Evaluation of Engineered Disc Replacements

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Introduction

Back and neck pain have become ubiquitous in modern society, and while the causes of spinal pain can be multifactorial, degeneration of the musculoskeletal components of the spine is a primary contributor. Although the intervertebral discs are the most commonly studied region of the motion segment with respect to degeneration and repair, it is becoming increasingly evident that degeneration of adjacent structures, such as the facet joints, vertebral endplates and paraspinal muscles, occurs concomitant with disc degeneration.[1] However, crosstalk between these adjacent components of the spinal motion segment during degeneration and following repair remains understudied, particularly in pre-clinical animal models. As a novel treatment strategy for end-stage disc and vertebral endplate degeneration, our group developed a composite, tissue engineered endplate and disc replacement (eDAPS).[2] We previously evaluated the eDAPS following *in vivo* implantation in a healthy goat cervical spine, however, the performance of the eDAPS in a degenerative spine, where pathology may be present in multiple spinal structures (as would be in human patients), remains unexplored. The purpose of the current study was to establish a model of spinal motion segment degeneration in the goat cervical spine via minimally invasive chemonucleolysis, quantifying alterations to disc and facet cartilage structure and mechanics, vertebral bone remodeling and trans-endplate small molecule diffusion.

Methods

With IACUC approval, four goats underwent a procedure to induce degeneration of the C2-C3 and C4-C5 intervertebral discs. Animals were anesthetized, and fluoroscopic guidance was utilized to identify the spinal levels of interest and place a 22G spinal needle within the central nucleus pulposus (NP). Discs were injected with either 200μL of 2U (n = 4 levels) or 5U (n = 4 levels) chondroitinase-ABC (ChABC), with doses based on our previous work in the lumbar spine (REF). The C3-C4 disc was utilized as a healthy control level. At 12 weeks post-ChABC delivery, animals underwent *in vivo* magnetic resonance imaging (MRI) at 3T to obtain T2 and T1 maps before and 30 minutes following intravenous administration of 0.1 mmols/kg of the non-ionic MRI contrast agent gadodiamide, to quantify diffusive transport into the disc.[3] Following euthanasia, vertebral body-disc-vertebral body motion segments and the accompanying facet joints were isolated. Motion segments were subjected to a biomechanical testing protocol consisting of 20 cycles of compression (0 to −100N) at 0.5 Hz, followed by 1 hour of creep loading at −100N. Mechanical properties (toe and linear region modulus, transition and maximum strain) were calculated from the 20th cycle of compression, and normalized to disc height and area, measured from the MR, as previously described.[4] Each facet articular surface was subjected to indentation testing using a 1mm indenter applying 15 minutes of creep loading at −0.1N. Mechanical properties including permeability (k_0), strain dependent permeability constant (M), tensile modulus, and compressive modulus were calculated from fits of the displacement-time curves using a Hertzian biphasic creep model.[5] For NP T2 values and disc mechanical properties, statistical differences ($p < 0.05$) were assessed via ANOVA with Tukey's post-hoc. Differences in pre- and post-contrast NP T1 relaxation times were assessed via paired t-tests. Differences in facet cartilage mechanics were determined via two-tailed Student's t-test.

Results

T2-weighted MRIs (Figure 1A), illustrated the spectrum of disc degeneration achieved by varying ChABC dosage, with 5U ChABC resulting in complete loss of signal from the NP at 12 weeks post-injection. This corresponded with a significant reduction in NP T2 relaxation times, indicative of reduced water and proteoglycan content (Figure 1B).[6] *In vivo* T1 mapping demonstrated a reduction in T1 relaxation times in the spinal tissues following contrast agent administration (Figure 1C). The reduction in NP T1 relaxation time from pre- to post- contrast injection was significant in the control and 2U discs, but was not in the 5U discs, suggesting that small molecule, trans-endplate diffusion

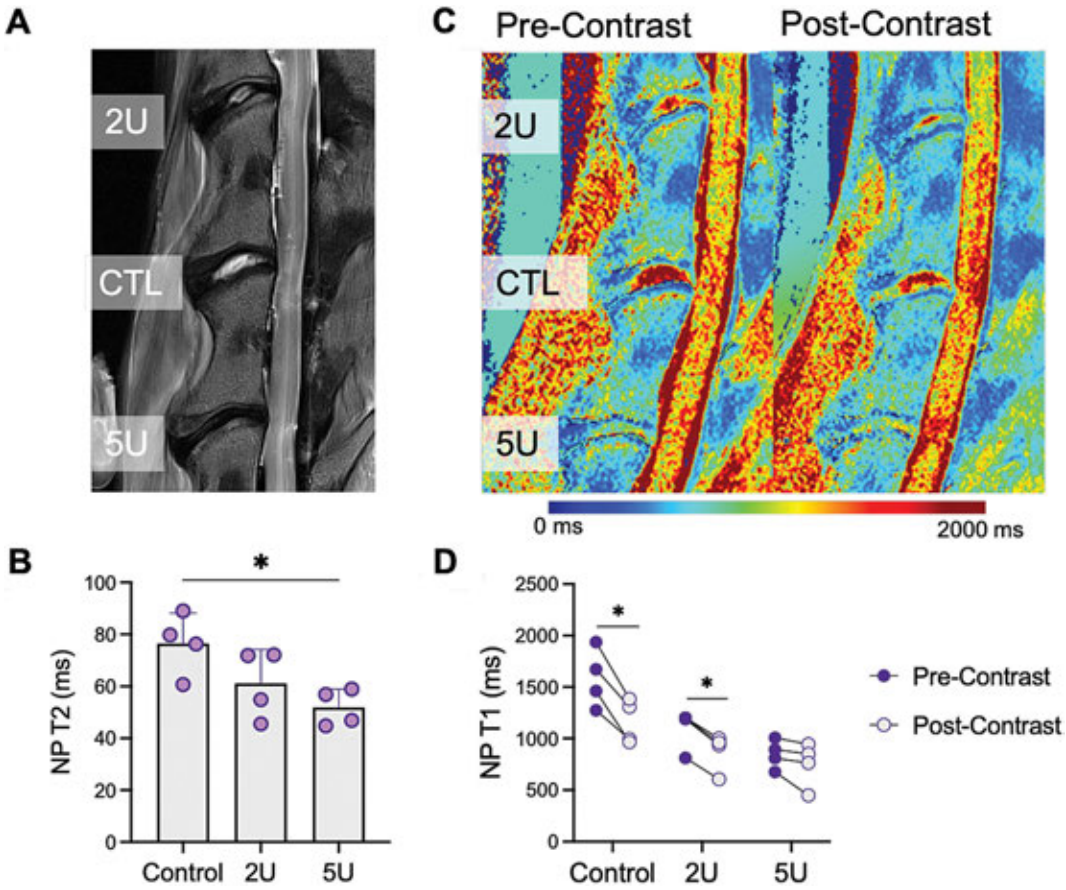

Figure 1. (A) Representative T2-weighted MRI of the goat cervical spine. **(B)** Quantification of T2 relaxation times in the nucleus pulposus. **(C)** In vivo T1 maps before and after gadodiamide administration from which (D) NP T1 relaxation times were calculated as a metric of contrast agent diffusion into the disc. (*=$p<0.05$).

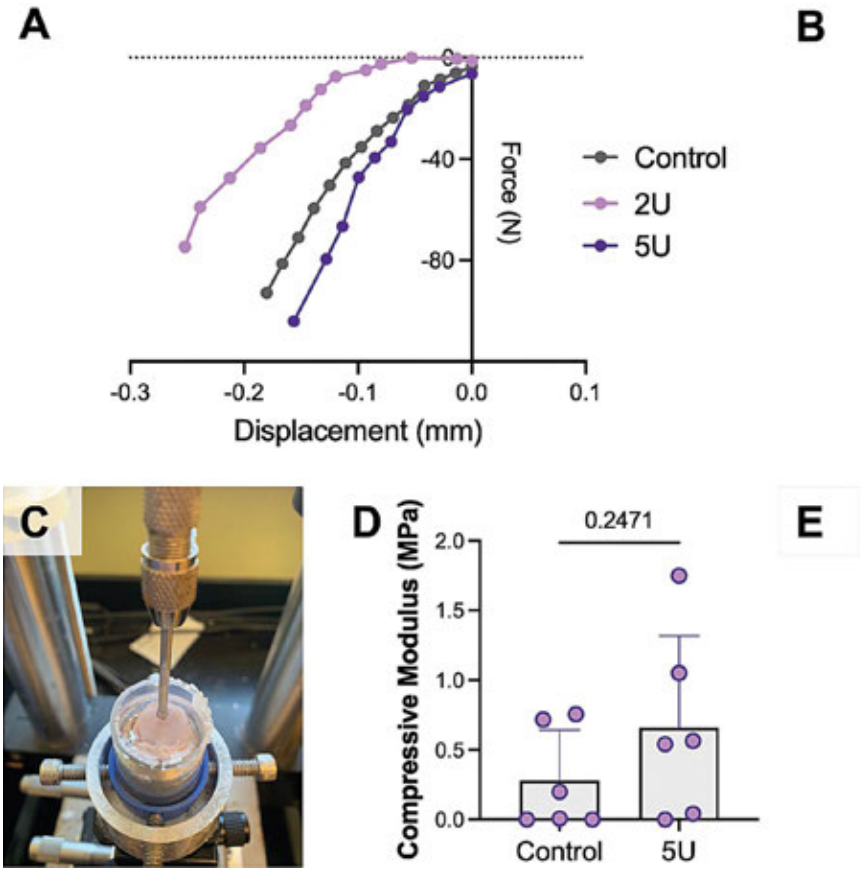

Figure 2. (A) Representative force-displacement curves from disc compressive mechanical testing, and **(B)** creep strain by group. **(C)** Facet cartilage creep indentation testing setup from which cartilage **(D)** compressive modulus and **(E)** the strain-dependent permeability constant, M, were determined.

was reduced in these severely degenerative discs (Figure 1D).[3] Motion segment force-displacement curves (Figure 2A) suggested a loss of compressive mechanical properties in the 2U discs, followed by stiffening in the 5U group. There was a trend towards increased creep strain in the 5U group compared to healthy controls (Figure 2B). Creep indentation testing of the facet articular cartilage adjacent to disc in the 5U ChABC group revealed trends towards altered cartilage mechanical properties, suggestive of degeneration, particularly an increase in compressive modulus and a reduction in the constant M, which dictates strain-dependent permeability (Figure 2C-E). No significant differences were detected in k_0 (Ctl: 0.0019 $mm^4/Ns \pm 0.001$; 5U: 0.0022 $mm^4/Ns \pm 0.001$) or tensile modulus (Ctl: 4.3 MPa ± 3.0; 5U: 2.6 MPa ± 1.0 MPa).

Discussion

In this study we established a large animal model where degeneration manifests across multiple components of the three-joint complex. Higher doses of ChABC resulted in more severe disc degeneration, as characterized via quantitative *in vivo* MRI. *In vivo* scanning also permitted quantification of small molecule diffusion into the disc; this was reduced in the 5U discs 12 weeks following ChABC injection. This may be due to remodeling of the vertebral bone and vasculature, as we have observed in a rabbit disc puncture model.[3] Mechanical properties of the disc, particularly creep strain, were altered substantially with degeneration, consistent with previous work.[4] These altered disc mechanics likely resulted in aberrant mechanical loading of the facet joints *in vivo*,[7] precipitating the articular cartilage degeneration suggested by altered cartilage indentation mechanical properties. The primary limitation of this study is its small sample size, making statistical differences in disc and facet cartilage mechanical properties difficult to detect given the heterogeneity in these measures. Ongoing work is focused on increasing this sample size and performing correlations across quantitative measures of disc and facet degeneration over longer durations to assess the progression motion segment degeneration.

Discussion

This large animal model provides a platform for studying the crosstalk between the discs and facet joints during degeneration at human length scales. Future work will utilize this model for the pre-clinical evaluation of a whole, tissue engineered disc replacement in a physiologically relevant degenerative scenario, in addition to other novel regenerative medicine approaches for the treatment of degenerative disc disease.

Acknowledges

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