Intervertebral Disc and Facet Cross-talk in a Rabbit Puncture Model of Spine Degeneration

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Introduction

28.5% of adult Americans suffer from lower back pain[1], which is frequently associated with intervertebral disc degeneration (IVDD). While IVDD has become a fairly well studied condition, there still remains a deficit in our understanding of how facet osteoarthritis (OA) contributes to degeneration of the whole motion segment. Facet osteoarthritis is a significant source of low back pain[2] and likely plays a critical role in the changing articulation of motion segments as they degrade[3]. Our previous work using a rabbit-puncture model of disc degeneration established the use of clinically relevant imaging and multi-scale characterization techniques to evaluate changes in the disc[4]. In this study, we expand on that rabbit model, linking IVDD with changes to facet cartilage to better understand the degeneration of the full motion segment in response to acute trauma.

Methods

Five New Zealand White male rabbits underwent surgery using an established IACUC approved rabbit-puncture model[4], in which 4 levels between L23 and L67 were punctured, two using a 16G needle and two using a 21G needle, leaving one adjacent level as a healthy control. At 10 weeks post-puncture, all animals were intravenously administered the small molecule, non-ionic MRI contrast agent, gadodiamide, 30 minutes prior to euthanasia[5]. Following euthanasia, experimental (n = 20) and control (n = 5) motion segments underwent coronal MRI T1 and T2 mapping to quantify small molecule diffusion across the endplate and disc T2 relaxation times, respectively. Motion segments from two rabbits (n = 8 experimental, n = 2 control) were subject to mechanical testing, consisting of 20 cycles of tension (21 N) and compression (42 N)[4]. Motion segments were subsequently scanned using μCT to quantify bone morphometry and then decalcified and processed for paraffin histology. Samples were sectioned in the sagittal plane and stained with Hematoxylin and Eosin and Alcian Blue/Picrosirius Red. Control discs from this study were pooled with controls levels from our previous work[4]. Corresponding facets (n = 16 experimental, n = 4 control) were dissected away from the motion segments for creep indentation mechanical testing[6] at one point on each articular surface. Facets (n = 9 experimental, n = 8 control) were processed for paraffin histology, sectioned in the sagittal plane at a thickness of 10 μm, and stained with Safranin-O and Fast Green. All disc and facet histology were scored using a 3-scorer consensus system according to the OARSI scoring system for rabbit cartilage[7] and the ORS Spine section intervertebral disc scoring system[8], respectively. All data was analyzed using a two-tailed T-test or a One-Way ANOVA with a Tukey's post-hoc test. Significance was defined as $p < 0.05$.

Results

With increasing severity of disc puncture, markers of disc degeneration became more pronounced at 10 weeks post-puncture. Composite T2 maps revealed a decrease in disc water content, primarily in the nucleus pulposus (NP) (Figure 1A). On histology, a loss of distinction between the nucleus pulposus and annulus fibrosus (AF) regions was observed as well as progressive reduction in NP size, loss of overall disc height, and increase in AF lamellar disorganization, culminating in an instance of NP herniation (Figure 1B-C). These structural changes yielded significant stiffening of the IVD in the 16G puncture discs, compared to controls and 21G puncture discs (Figure 1D). A reduction in small molecule transport into the NP was also measured, particularly in 16G punctured discs (Figure 1E) and was accompanied by increased vertebral endplate bone volume fraction (Figure 1F), primarily driven by trabecular thickening. Corresponding facets showed early signs of cartilage degeneration. Facet cartilage indentation testing suggested an increase in compressive moduli alongside a decrease in cartilage permeability (Figure 2A). Surface fibrillations and irregularities were observed in both experimental groups. Notably, facets corresponding to 21G punctured discs experienced a loss of Safranin-O staining in the superficial zone and a loss of chondrocyte

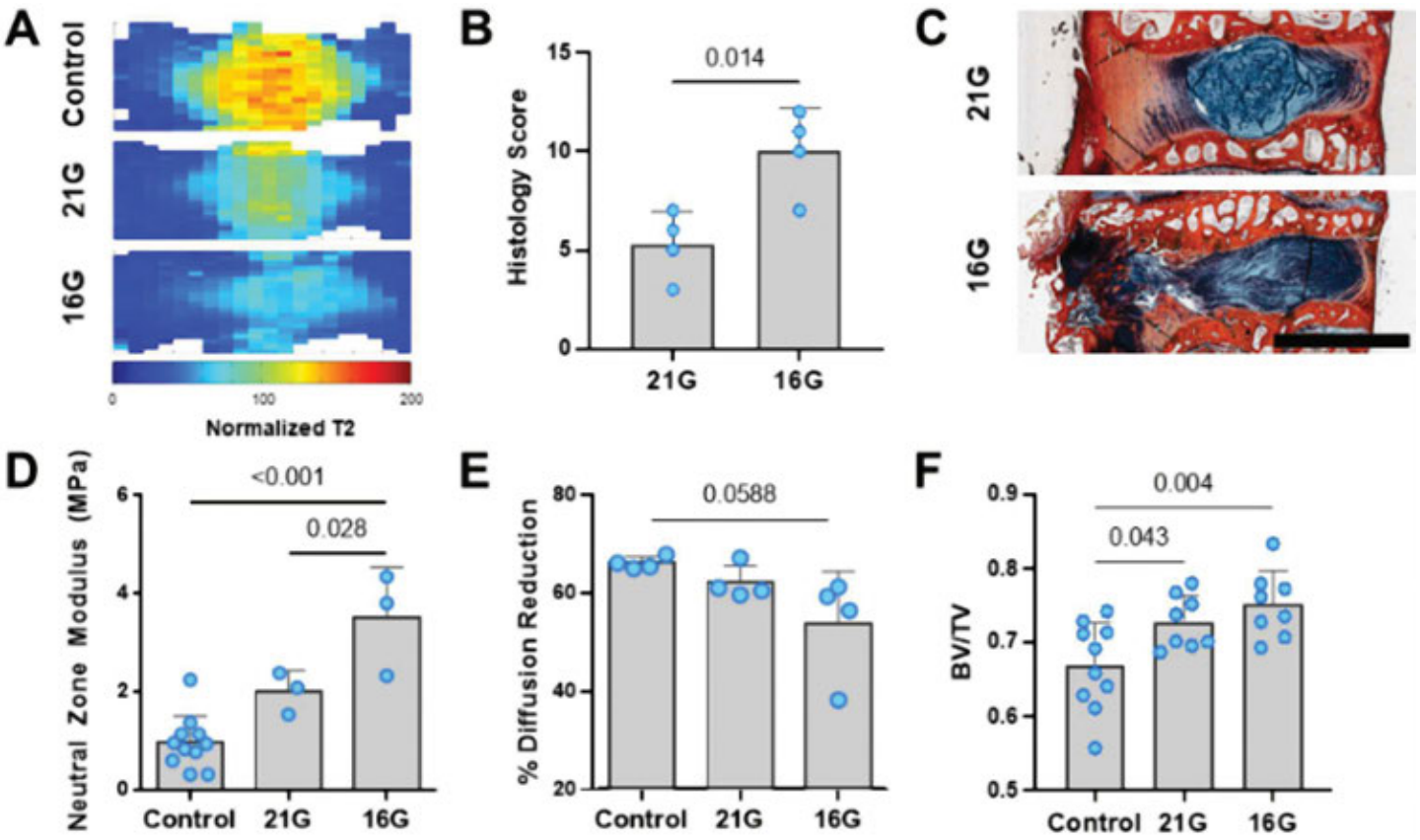

Figure 1. For control and puncture intervertebral discs: **(A)** Average T2 MRI maps, **(B)** consensus ORS Spine histology scores, **(C)** representative Alcian Blue/Picrosirius Red histology (scale bar = 4 mm), **(D)** neutral zone modulus from compression-tension mechanical testing, (E) small molecule diffusion reduction across the endplate, and (F) bone volume fraction as determined by ΔCT.

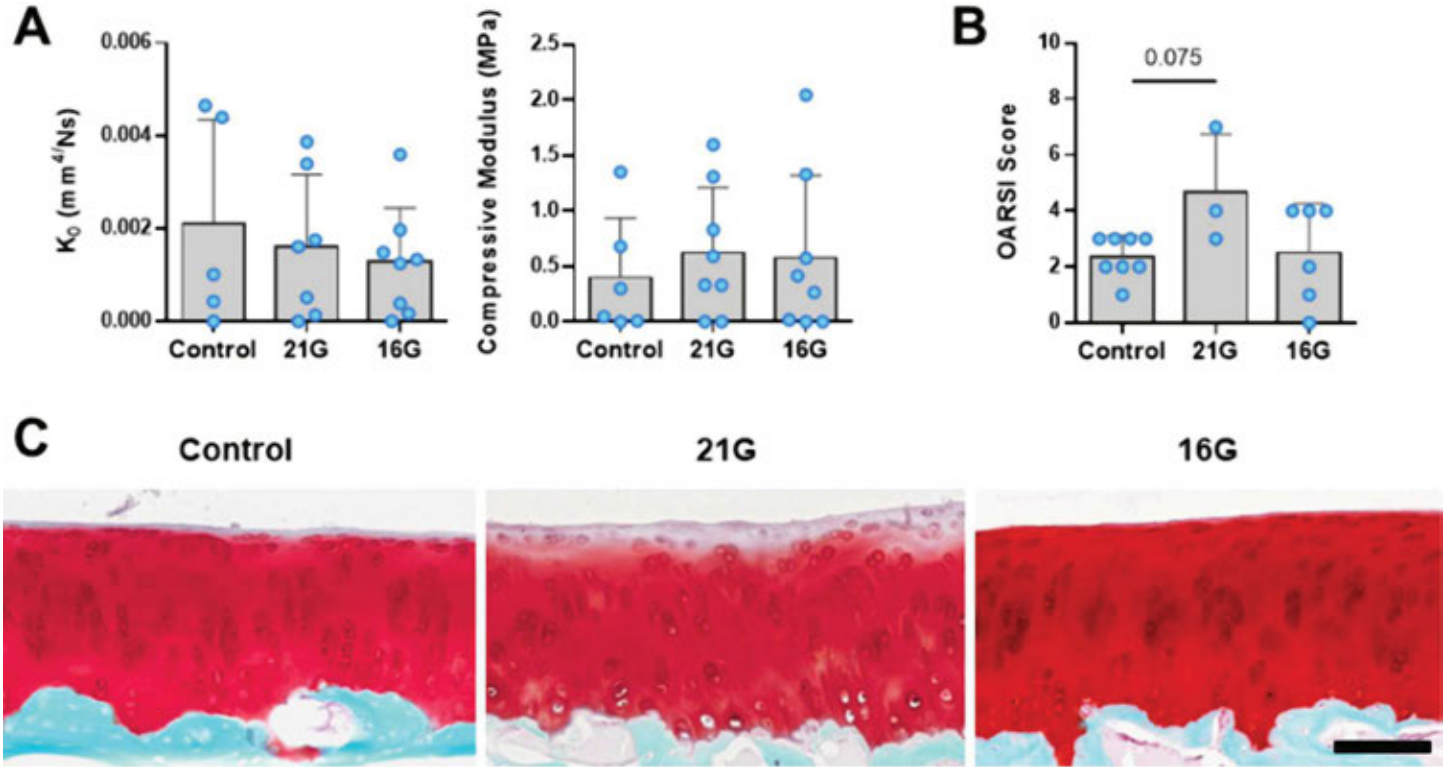

Figure 2. For control facets and facets corresponding to punctured discs: **(A)** Permeability and compressive modulus from creep indentation mechanical testing, **(B)** consensus OARSI scores, and **(C)** representative Safranin-O, Fast Green histology (scale bar = 100 mm).

density and columnar organization when compared to both control facets and 16G puncture facets (Figure 2B-C).

Discussion

After 10 weeks, intervertebral discs followed an increasingly severe path of degeneration with increasing diameter of needle puncture. Disc degeneration was characterized by a progressive loss of water in the NP, which is likely linked to a loss of proteoglycans and an increase in matrix density. Increased endplate bone density adjacent to the punctured discs also led to a decrease in small molecule transport into the disc, which, at later time points, may further exacerbate the degenerative cascade. Overall, the adjacent facets in both groups underwent minimal remodeling in comparison to the discs. However, histologically, while there was an increase in facet OA between the control facets and 21G puncture facets, there was little change in the 16G puncture facet cartilage.This may be linked to the stiffening of the anterior compartment of the motion segment observed in this group. As the disc becomes stiffer, through both the formation of osteophytes anteriorly and the deposition of fibrotic matrix, the facets are progressively offloaded. The significant stiffening of 16G discs compared to 21G discs may be shielding the facets from cumulative, severe loads, minimizing the progression of cartilage osteoarthritis as is seen in offloading studies in the knee[9]. Overall, 10 weeks does not seem to be a sufficient time scale for facets to respond to the changing mechanical and biochemical environments of the corresponding discs. Longer study durations would likely elucidate the progression of facet deterioration and how that progression affects the entire motion segment.

Significance

This work links our knowledge of IVDD with facet osteoarthritis, allowing for a better understanding of the progression of whole motion segment degeneration, which can aid in informing the development and evaluation of novel regenerative strategies for spinal degeneration.

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