

Mechanical and Biochemical Consequences of Needle Puncture Injury in the Mouse Caudal Disc

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INTRODUCTION Models of acute annulus fibrosus (AF) injury consistently produce nuclear depressurization and biological remodeling similar to that of human disc degeneration. These methods are easily implemented, provide repeatable results and have controlled specificity in comparison to global knockout and spontaneous development models. AF injury studies have been conducted in many species including the sheep, rabbit, and rat¹⁻⁴. In addition, the mouse caudal disc is a proposed model, as it degenerates as a result of needle puncture injury, displaying changes consistent with those in humans⁵. The primary function of the disc is mechanical - transferring loads, dissipating energy and facilitating joint mobility. Evaluating disc mechanical properties is therefore important for understanding how functional changes can be targeted by potential therapeutics. However, limited mechanical data for puncture-initiated degeneration is available. **The objective of this study was to quantify the acute and long term effects of needle puncture injury on mouse caudal disc mechanical properties, disc height and glycosaminoglycan (GAG) content.**

METHODS

Surgery: With institutional IACUC approval, retired breeder mice were allocated to two post-surgical time points: zero weeks (n=10) and eight weeks (n=5). The (caudal) C6/C7 and C8/C9 discs were either exposed and punctured with a 29G needle ($\approx 65\%$ disc height) or exposed as an intact sham control. The needle was clamped and inserted 1.75 mm to ensure full penetration of the adjacent annulus.

MicroCT: Caudal spines were removed and imaged *en bloc* at an isotropic 21 μm resolution. Disc height (h) and polar moment of inertia (J_z) were measured using a custom Matlab program after converting 3D reconstructions across the disc space to image stacks.

Mechanical Testing: Spines were then separated into motion segments, equilibrated in PBS and 4°C overnight and tested in tension/compression (20 cycles: +0.5 N, -1.5N, 0.025 Hz), creep (-1.5 N, 1 hour), and torsion (10 cycles: $\pm 8^\circ$, 0.05 Hz) on an Instron 5542 mechanical testing system equipped with a custom torsional testing device. The device is composed of a stepper motor in series with a torque cell and two digital cameras to track the extension and rotation of each motion segment (Fig.1). Total range of motion (ROM), creep displacement, and torsional stiffness were calculated from the raw data. All mechanical parameters were normalized to disc geometry: total ROM and creep displacement were multiplied by 1/h while torsional stiffness was multiplied by h/J_z .

GAG Content: Following mechanical testing, each disc was dissected from the adjoining vertebral bodies using a cryostat microtome. A 0.75 mm diameter biopsy punch was used to isolate the nucleus pulposus which was digested in papain. GAG content was then measured using the DMMB assay.

Statistics: Differences in properties between intact and injured discs, and between 0 and 8 weeks were established using ANOVAs and posthoc Tukey's tests ($p < 0.05$).

RESULTS Mean disc height was 19% lower for injured discs ($p < 0.05$) compared to intact controls after eight weeks (Fig. 2). Disc height for shams also decreased after 8 weeks, but not significantly. GAG content was also lower for injured discs (19%) and shams (17%) after 8 weeks compared to day 0 intact controls, but not significantly for either (Fig 2). Total ROM was 32% greater for injured discs after 8 weeks compared to day 0 intact controls ($p < 0.05$). ROM for sham treatment was also significantly greater after 8 weeks. Creep displacement was 34% greater for injured discs after eight weeks ($p < 0.05$) than day 0 intact controls. There were no differences in torsional stiffness intact/sham and injured discs at 0 or 8 weeks

CONCLUSION In this study an acute AF injury was used to create degenerative changes in the mouse caudal disc. Reduction in GAG content (nonsignificant), which has been extensively correlated to degenerative grade, was measured as well as a corresponding decrease in disc height. For the zero week group, the 29G needle did not produce differences between control and punctured discs, contrasting rat tail needle puncture studies by Elliott⁶ and Michalek⁷ but similar to another by Hsieh⁸. Increases in ROM and creep displacement at 8 weeks are consistent with the response of the rat caudal disc to GAG depletion by

chondroitinase ABC injection⁹. It has been suggested that the presence of sufficient mechanical perturbation is required for degenerative changes to take place⁸. Consistent with this theory, this study demonstrates that minor changes in mechanical properties following needle puncture *ex vivo*, lead to moderate degenerative changes *in vivo*. This suggests that the biological response following puncture injury is more detrimental to disc mechanics than the puncture itself. However, because there were no differences between sham and treatment groups, an examination of later timepoints is required to deduce whether these changes are a result of needle puncture and not a transient inflammatory response. Future work will consist of evaluating larger needle sizes and later timepoints.

SIGNIFICANCE A mouse model of disc degeneration is a valuable tool given the number of available molecular probes and genetic techniques with high temporal and tissue-specific resolution, as well as the logistical advantages over large animals like small cage sizes and inexpensive daily maintenance. The primary function of the disc is mechanical, thus evaluating disc mechanics is important to understanding how functional changes can be targeted by potential therapeutics.

ACKNOWLEDGEMENT The authors would like to thank Dr. Joseph Sarver for the digital texture tracking algorithm. This study was funded by NIH/NIAMS. **REFERENCES** (1) Osti+ *Spine* 1990, (2) Sobajima+ *Spine* 2005, (3) Ulrich+ *Spine* 2007, (4) Miyamoto+ *Spine J* 2006, (5) Yang+ *J Pathol* 2009, (6) Elliott+ *Spine* 2008, (7) Michalek+ *Eur Spine J* 2010, (8) Hsieh+ *Spine* 2009, (9) Boxberger+ *Spine* 2008

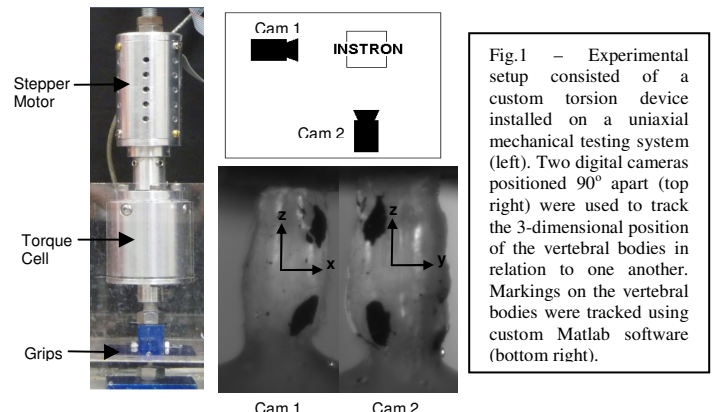


Fig.1 - Experimental setup consisted of a custom torsion device installed on a uniaxial mechanical testing system (left). Two digital cameras positioned 90° apart (top right) were used to track the 3-dimensional position of the vertebral bodies in relation to one another. Markings on the vertebral bodies were tracked using custom Matlab software (bottom right).

