Introduction – Needle puncture injury models are widely used to study intervertebral disc degeneration and associated healing and repair processes [1-4]. Rabbit and rat caudal models have been studied extensively [1,2,3], and more recently, the mouse caudal disc has also been proposed as a potential model [4]. Few disc injury studies, however, have quantified baseline mechanical changes, which are critical in the initiation of the degenerative cascade. A recent review suggested that puncture with a ratio of needle diameter to disc height greater than 40% would lead to disc degeneration [5]; however, this was based primarily on outcomes from in vivo studies. It is unknown to what extent the degenerative changes observed in in vivo studies result directly from altered mechanical properties following the initial injury. Moreover, data for the direct effects of needle injury are inconsistent: in a rat disc puncture study a 33 gauge needle in the lumbar (26% of disc height) had no mechanical effect [6], while in another study a 30 gauge needle in the caudal (15%-55% of disc height) significantly decreased dynamic stiffness [7]. Thus it is unknown whether the lumbar and caudal discs behave differently following needle injury. The objective of this study was to investigate the direct effects of intervertebral disc needle puncture injury on mouse lumbar and caudal disc mechanical function. We hypothesized that 31 gauge needle injury in the lumbar (62% of disc height) and caudal (54% of disc height) would significantly alter mechanical properties in axial compression.

Methods - Institutional IACUC approval was obtained for all animal experiments. Six month old retired breeder mice were euthanized, and lumbar and caudal spines were dissected out and laterally imaged using a fluoroscope for disc height measurements. Lumbar L1-2, L3-4, and L5-6 (without posterior elements) and caudal C6-7, C8-9, and C10-11 bone-disc-bone segments were prepared. One lumbar and one caudal level from each animal were each randomly assigned to either needle injury (n=6) or intact control (n=7). Following overnight equilibration in PBS, samples were mounted in custom grips; for the injured group, a custom 31 gauge needle was inserted into the lateral annulus fibrosus (AF) to a controlled depth corresponding to half the disc width. Testing consisted of 20 cycles at 0.05mm/s between −1.5N to 1.0N (lumbar, strain −18% to 22%) and −1.5N to 1.5N (caudal, strain −22% to 36%). These ranges were selected to fully develop the compression and tension regions of the loading curve without causing damage. A 1.5s step load was then applied to −1.5N and held for a 1 hour creep test. The 20th cycle was analyzed using a custom Matlab program to determine the zero point and calculate the neutral zone (NZ) stiffness, NZ length, and compressive stiffness [6]. The creep response was fit to a 5 parameter rheological model consisting of an elastic stiffness and two exponential decays defined with a time constant and stiffness [8]. Following testing, the disc was cut axially and whole disc and nucleus pulposus (NP) area were calculated; all mechanical parameters were then normalized by disc geometry [9]. Differences between the punctured and intact groups were examined using unpaired t-tests (p≤0.05). The efficacy of the needle injury was examined histologically: following fixation and decalcification, mid-sagittal sections were cut from paraffin embedded samples and stained with picrosirius red and alcian blue.

Results - Height and area were not different among the levels studied for either the lumbar (height 0.42 ± 0.05 mm, area 2.24 ± 0.41 mm²) or caudal (height 0.48 ± 0.06 mm, area 2.62 ± 0.37 mm²) discs. The ratio of NP to disc area was 25% in the caudal NP, which was significantly larger than the 16% in the lumbar. Histology confirmed that needle injury disrupted the AF (Fig. 1).

In the lumbar spine, needle injury did not significantly affect normalized NZ or compressive stiffness (Fig. 2). The creep parameters and creep displacement (Fig. 2) were unchanged (p>0.05). The normalized NZ length decreased by 24% (trend, Fig. 2). In the caudal discs, needle injury did significantly affect the axial mechanics; normalized NZ length significantly decreased by 33% after injury (Fig. 2), normalized NZ stiffness increased, and there was no change in normalized compressive stiffness (Fig. 2). The injury also affected caudal discs in creep, with a 33% decrease in normalized creep displacement (Fig 2) and changes in several model parameters (not shown), resulting in an average creep response following needle injury (Fig 3).

Discussion - This study shows that needle puncture injury has little direct effect on the elastic response in mouse lumbar and caudal discs compared to the large effect following rat NP glycosaminoglycan depletion [6] and in vivo mechanical changes observed 8 wks after rabbit disc injury [1]. Indeed, the trends observed here (decreased NZ length) are opposite to increased NZ length observed with glycosaminoglycan depletion [6]. This suggests that mechanical changes observed in in vivo injury models result from downstream biological and structural changes and not a direct consequence of the initial injury. For example, the degenerative process in injury models may be driven by medium- or long-term wound healing or by a cellular response to local altered loading. This study did however show significant effects of needle injury in the viscoelastic creep response, but only in the caudal disc. This is consistent with dynamic modulus changes described previously [7]. Additionally, we have previously demonstrated mechanical differences between mouse lumbar and caudal disc NZ and creep response that are consistent with this study [10]. These may be due to anatomical differences between lumbar and caudal discs (e.g., larger caudal NP). While the initial injury effects are different between the lumbar and caudal disc, it remains to be determined whether these are large enough to differentiate the biological response in vivo, or whether they would be obfuscated by the large glycosaminoglycan and other disc changes that occur with degeneration. The direct mechanical effects of needle injury reported here establish an important baseline for evaluating subsequent progression of degeneration in in vivo models.

Acknowledgement – This study was funded by NIH/NIAMS.


Poster No. 1571 • 56th Annual Meeting of the Orthopaedic Research Society