INTRODUCTION

Many potential treatments to regenerate or repair degenerated intervertebral discs require needle delivery systems. However, there is evidence that insertion of 27G and 28G needles into the annulus fibrosus or nucleus pulposus (NP), without herniation, leads to mild and moderate degeneration over time in rabbit and sheep models [1,2]. The early mechanical and biological response to needle puncture in a large animal model is not clear. We hypothesized that significant changes in disc structure, mechanics, and cellular response would be present within one week after small or large gage needle puncture in a bovine organ culture model.

METHODS

Bovine tails obtained from a local abattoir within 4 hours post-mortem were randomly assigned to an unpunctured control group (N=10), and one of two needle puncture groups (small = 25G syringe, N=11; large = 14G syringe, N=12). Musculature surrounding the intervertebral disc was removed. Caudal discs c3-c5 were punctured using a postero-lateral approach through the annulus taking care to only puncture as far as the NP. Discs were removed from vertebral endplates and initial disc heights, diameters, and wet weights were measured prior to culturing. Specimens were then placed in an organ culture chamber and incubated in standard culture conditions at 37°C and 5% CO2 under a 0.2 MPa static load as previously described [3]. Media was continuously circulated through the chamber (1.1mL/min) and changed every 2 days.

After an initial 12 hour equilibration period under 0.2 MPa [3], chambers were individually attached to an incubator-housed dynamic loading device. A one minute test (0.2-0.4MPa, 1Hz) was applied to obtain a pre-loading dynamic nominal modulus for all groups, followed by one hour of dynamic loading (0.2-1.0MPa, 1 Hz), and finally a repeat of the one minute test to obtain a post-loading nominal dynamic modulus. Change in disc height over the one hour loading period was assessed from displacement data obtained through the loading device. After the mechanical intervention, 0.2 MPa static load was again applied to allow at least 12 hours of recovery. Loading occurred once per day (5 times) during the 6 day culture period.

Glycosaminoglycan (GAG) content released to the media was assayed using the dimethylmethylene blue (DMMB) assay and was normalized to initial wet weight of the intact disc. Regional water contents for tissue samples in the outer and inner annulus (OA, IA), and NP were determined for each group by comparing wet and dry weights.

Cell viability was assessed as previously described [3]. Live and dead cells were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT thiazole blue, Sigma Aldrich, St. Louis, MO) and ethidium homodimer-1 (Molecular Probes, Eugene, OR), respectively. Samples were sectioned into 10 μm thick slices and images of each section were obtained at 20x under fluorescent (ethidium) and brightfield (MTT) conditions. Tissue samples were also fixed in formalin, embedded in paraffin, and stained with alcin blue (proteoglycans), picosirius red (collagen), and Weigert's hematoxylin (cell nuclei) for histologic appearance.

For all quantitative variables, ANOVA with Bonferroni-adjusted post-hoc comparisons were performed using p<0.05 significance level.

RESULTS

The nominal dynamic modulus was significantly affected by needle puncture (P=0.009), with average values for the large needle group being significantly lower than for control (Figure 1, P=0.023). No significant differences existed between small and large needle groups, nor small needle and control groups (P>0.19). Needle puncture also affected the height lost during the one hour load cycle, with significant differences between needle puncture and control groups (Figure 1, P<0.006). The amount of GAG released to the media and regional tissue water contents were not significantly affected by needle puncture (P>0.125).

Cell viability was maintained in discs although localized cell death was observed in the area adjacent to the needle tracks. Histology also revealed annulus fiber disruption, and increased cell number and remodeling in the NP of the large needle group (Figure 2).

DISCUSSION

Localized disruption in the disc tissue from both small and large needle puncture was demonstrated to rapidly compromise disc structure and mechanical properties consistent with our hypothesis. Evidence of a cell mediated response was also present, particularly in the NP region of the large needle group with evidence of matrix remodeling and increased cell number. It is notable, however, that disc cell viability generally remained high (with the only exception in the area immediately adjacent to the needle track), and that needle puncture did not affect GAG released from the disc or water content after recovery. Results indicate a relatively minor disruption in the disc from needle puncture has very rapid mechanical and biological consequences with important implications for the use of needle puncture in discography and stab models of degeneration. Results also suggest early matrix disruption might have profound mechanical implications that would be difficult to detect from traditional MRI techniques.


Figure 1: Average ± SEM pre-load nominal dynamic modulus (top) Average ± SEM height loss during 1 hour dynamic loading (bottom)

Figure 2: Histology images of the OA (left) and IA (middle) at 2.5X (scale = 1mm), NP (right) at 20X (scale = 0.4mm), control discs on bottom row & large needle punctured discs on top row.