Introduction: Small animal models of the intervertebral disc (IVD) are of increasing importance for investigating remodeling and degeneration. Composition and structure of the IVD annulus fibrosus and nucleus pulposus define elastic and viscoelastic material behaviors of the disc. The main aim of this work is to refine structure-function relationships between biomechanical behavior of the IVD and its biochemistry by inducing changes in collagen and proteoglycan structure and content in order to simulate degeneration. Additionally, due to their frequent use during in vivo studies, rat lumbar and caudal disc biomechanical behavior was compared. Specifically, we hypothesize that 1) proteoglycan and collagen degradation will decrease compressive stiffness while collagen crosslinking will increase it and 2) lumbar and caudal discs will exhibit similar viscoelastic and elastic behavior under in vivo resting stress.

Materials and Methods: One lumbar level and three caudal levels were harvested from each of 17 Sprague Dawley rats and potted. Caudal motion segments were randomly assigned to one of five groups (n=10): fresh control, PBS soaked control, proteoglycan degraded (chondroitinase ABC), collagen degraded (collagenase) and cross-linked (Genipin). Potted motion segments were pinned during chemical treatments to prevent axial swelling and soaked for 12 hours. Mechanical testing was conducted on an Enduratec testing machine in PBS and consisted of 5 stages: rehydration-equilibration, compression-tension loading, quasi-static compression, frequency sweep, and creep. Data at each stage was analyzed with Matlab: a trilinear fit was applied to the tension-compression test, quasi-static stiffness was obtained from the slope of the compression curve, dynamic stiffnesses and phase angles were calculated from the frequency sweep and a stretched exponential was fit to the creep data.

Results: In the trilinear fit, Genipin caused a significant increase in neutral zone stiffness, while also decreasing the neutral zone length. Collagenase caused a significant increase in neutral zone length (Figure 1).

In creep, disc height change and the apparent time constant, $\tau_B$ were significantly lower for the collagenase treated group (Figure 2). In the frequency sweep showed an increase in stiffness with frequency for all groups. There was also a significant increase in stiffness with soaking for all groups, and the collagenase treated group was significantly stiffer than the other soaked groups.

Quasi-static stiffness was not impacted by any of the chemical interventions. Trilinear fits in compression and tension similarly showed minimal differences between groups.

No significant differences were found between lumbar and caudal motion segments in quasi-static stiffness, tensile or compressive stiffness, complex stiffness, phase angle, or height change during creep. The lumbar disc was found to be stiffer and to have a shorter neutral zone length and also to have a shorter apparent time constant in creep.

Discussion: Several enzymatic treatments were applied to rat motion segments to establish structure-function relationships. The most important result was that collagen degradation and crosslinking had the largest effects on elastic and viscoelastic behaviors consistent with the hypotheses.

Elastic sensitivity was most strongly illustrated by the changes in neutral zone due to different chemical interventions. Neutral zone stiffened and decreased in length with Genipin, which may be explained by crosslinking limiting the sliding of fibers (and consequently neutral zone laxity), decreasing fiber crimp and increasing fiber recruitment at lower displacements. Conversely, collagenase increased the neutral zone length by weakening the connections between fibers. Results also suggest a certain robustness in IVD compressive behaviors beyond the neutral zone since quasi-static and compression-tension stiffness were relatively insensitive to the chemical interventions.

Viscoelastic sensitivity was best demonstrated by differences in the apparent time constant $\tau_B$, and the complex stiffness. $\tau_B$ was decreased with collagen degradation which suggests more rapid solid matrix viscoelasticity. Similarly, the complex stiffness increased with collagenase digestion due to an initial compaction as measured during the rehydration-equilibration phase. The PBS and chondroitinase ABC digestion groups had similar results for all mechanical behaviors, suggesting that hydration of the disc dominated proteoglycan degradation, in contrast to the hypothesis. Additionally while some neutral zone sensitivity between rat lumbar and caudal discs were detected, as previously reported (1), most mechanical parameters were quite similar in compression.

In conclusion, elastic and viscoelastic IVD properties in compression were sensitive to the content and structure of collagen in the IVD and less sensitive to proteoglycan digestion. Specific structure-function relationships found may provide a model for interpreting biomechanical changes observed in animal modeling studies, and underscore the need to maintain and/or repair collagen integrity in IVD health and disease.

References:

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