Changes in the Matrix Composition of the Degenerating Human Intervertebral Disc and their Relative Contributions Towards Tissue Mechanical Behavior

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INTRODUCTION

The intervertebral disc (IVD) matrix consists primarily of collagen, proteoglycans, and water [1]. With aging and degeneration, the IVD matrix can undergo changes in these respective matrix components that culminate in changes in the tissue mechanical behavior. We have previously demonstrated that the degenerating intervertebral disc shows a decline in the elastic and viscoelastic properties in both the annulus fibrosus (AF) and the nucleus pulposus (NP) [2]. Specifically, the degenerative status is associated with AF elastic stiffness and NP viscoelastic damping. In this study, we aim to define the relative contributions of matrix component changes to the associated changes in the mechanical behavior of the IVD.

METHODS

11 lumbar spines (T12/L1 – L5/S1) were collected from the Willed Bodied program at the University of California, San Francisco (age/gender: 63M, 66M, 72F, 78F, 79F, 82F, 86F, 87F, 88M, 91M, 99M; Mean age: 82.5 ± 10.2). The cross-sections of the lumbar disc were visually categorized into Thompson grades 1 to 5 [3]. The grading was corroborated with fluoroscopic images. The discs were then separated into AF and NP sections, which then underwent various biochemical assays to determine matrix composition. Water content was determined by freezing the tissue before and after a 24-hour vacuum desiccation period. The tissues were then digested by papain digestion, aliquoted for assays, and followed by 6N HCl acid hydrolysis. Proteoglycan content was measured from the papain digests using a dimethyl-methylene blue (DMMB) dye-binding assay normalized to a commercially available chondroitin sulfate standard and divided by the collagen content. Collagen content was measured from the acid-hydrolysates using a chloramine T absorbance assay that measured the hydroxyproline content and divided by the dry tissue mass. Collagen crosslinking via advanced glycation end-products (AGEs), was quantified by measuring the autofluorescence of the acid hydrolysates at 370nm emission and 440 nm excitation and divided by collagen content. The measurements of tissue-level mechanical properties were described elsewhere [2] but used here for the regression analyses to determine the relationships between matrix composition and mechanical behavior of the tissue. These relationships were determined using Pearson’s correlation.

RESULTS

The sample collection and grading of the discs yielded in 4-9 discs (either AF or NP) per degeneration grade. The proteoglycan, collagen, AGEs and water content all showed marked changes with degeneration (Fig 1). Correlation analyses (Table I) revealed that AF indentation modulus was most greatly influenced by collagen content (R = 0.56) and the amount of advanced glycation end-products (R = 0.62); AF tan δ correlated most with the water content (R = 0.47), which in turn is correlated with proteoglycans (R=0.77) and AGEs (R= 0.48). In the nucleus (Table II), the indentation modulus was weakly influenced by proteoglycans (R = 0.12) and advanced glycation end-products (R = 0.22), while NP tan δ is correlated with the advanced glycation end-products and water content.

DISCUSSION

Disc degeneration creates a multitude of changes in the disc matrix that affects the disc mechanical behavior. These results suggest that the elastic behavior of the AF is dependent upon the collagen content as well as the amount of collagen crosslinking by AGEs; the viscoelastic behavior of the AF is influenced by water content and proteoglycans. The NP elastic behavior is influenced by AGEs and proteoglycans, while the NP viscoelastic behavior is mostly influenced by AGEs and water content. These changes in the matrix are inter-related and can be influenced by other matrix components.

Advanced glycation end-products are associated with changes in water content and tissue material behavior, suggesting that along with proteoglycans, AGEs can play a significant role in the deterioration of the IVD tissue mechanics during degeneration. AGEs may also affect the effectiveness of proteoglycans in the tissue’s ability to retain water [4]. Thus, the therapeutic strategies must be directed towards addressing the multiple changes that are occurring within the degenerating disc.

REFERENCES


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