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Mechanical profiling of intervertebral discs

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Abstract

Despite recent advances in imaging diagnostic technology and additional treatment options our ability to prevent or inhibit discogenic back pain has not drastically improved. The challenge of linking early degenerative patterns to dysfunction and pain remains. Using a novel material testing device designated the Tissue Diagnostic Instrument (TDI) we measured the local stiffness and strain energy absorption in the radial direction of 13 intact intervertebral discs; effectively generating a mechanical profile of each disc. Prior to measuring mechanical properties, an MR image was taken of each spine segment and the discs were radiologically scored according to the Pfirrmann scale. After testing, a sagittal portion of each L1-L2 disc was excised from each of four spines for histology. No significant correlations were found between Pfirrmann grade and mechanical data. However, polarized light microscopy images of disc sections indicated correlations between local tissue modulus measured with the TDI and the clarity and density of lamellar striations.

Introduction

Intervertebral disc injury and degeneration account for a disproportionately large fraction of health care costs in the United States. Inflation adjusted expenditures associated with treating back pain have increased 65% from 1997 to 2005, yet the effectiveness of treatments has not significantly improved.⁹ Although many promising therapeutic approaches have been proposed, ranging from regeneration via stem cells^{6,7} to implantation of synthetic components¹⁰, many fundamental issues have yet to be addressed. Linking degenerative tissue features to alterations in mechanical properties is central to understanding disc disease mechanisms.

Mechanistically, the intervertebral disc is analogous to a thick-walled fiber-reinforced pressure vessel absorbing strain energy and transmitting loads down the spinal column. The exterior annulus fibrosus is primarily a tensile member; it provides structural stability as the vessel wall with collagen fibril families layered in concentric lamellae of alternating orientation (between $\pm 45^\circ$ and $\pm 65^\circ$ off spinal axis).³ The nucleus pulposus, rich in hydrophilic proteoglycan, serves to imbibe water and pressurize the disc; distributing loads to the annulus and vertebral endplates while maintaining disc height. With degeneration, the

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graded properties of the disc change and this can be seen morphologically, biochemically and mechanically.^{2,8,15} Since disc degeneration is known to be associated with reduced lamellar order and a decreased ability to morphologically differentiate between annulus and nucleus¹⁴, a mechanical evaluation of the graded properties of the disc may be a useful tool for understanding changes in microstructure.

The Tissue Diagnostic Instrument (TDI) was modified from the Bone Diagnostic Instrument⁵ so as to measure soft tissue mechanical properties at a spatial resolution on the order of 1 mm. This device offers a simple quantitative way of resolving local elastic and viscous components of the tissue. It was hypothesized that the TDI could detect changes in the viscoelastic properties across a disc; effectively generating a mechanical profile. It was further hypothesized that features of these mechanical profiles would correlate with Pfirrmann grading based on MRI and with histologic observations of lamellar structure. Correlating local measures of disc mechanical properties with collagen structure and proteoglycan content will be essential to defining degenerative patterns and potentially enable early diagnostic methods for the mitigation of disease.

Methods

Magnetic resonance imaging

A total of four cadaveric human lumbar spines ranging in age from 32-72 were harvested and frozen (-50°C) at autopsy. A total of 13 discs were studied: discs L1-L5 were tested in three spines, and disc L1-L2 was tested from the remaining spine. The intact segments were imaged on a 3 Tesla GE Excite Sigma whole body MR scanner (General Electric Medical Systems, WI) using a GE 8 channel phase array knee coil. The image protocol was composed of a T2 weighted fast spin echo sequence. Pfirrmann scoring was performed by an experienced radiologist blinded to the mechanical testing protocol.¹¹ Pfirrmann scoring is largely based on apparent water content, which in turn, correlates with proteoglycan content.

TDI mechanical testing

The TDI consists of a force generator that reciprocates a 0.33 mm diameter test probe within a 23-gauge needle (reference probe) while simultaneously quantifying test probe axial forces and displacements. The device generates force versus displacement hysteresis curves (Figure 1c,1d). The slope of the force vs. displacement hysteresis loop (specifically, the slope of a least squares fitted line through the data; units of N/m) is an indication of local tissue stiffness in the probe axial direction; while the area contained within the hysteresis loop is a measure of viscous energy dissipation, or strain energy absorbed upon a load cycle (units of J).⁴

For each of the 13 intervertebral discs in this study, maximum medial-lateral diameter was measured to the nearest millimeter with calipers. The probe was inserted in the medial-lateral direction along the disc mid-height to depths of 10, 20, 30, 40 and 50% of medial-lateral diameter (Figure 1). At each successive insertion depth, ten hysteresis cycles were recorded. This procedure was repeated for 3 separate insertions on each of the thirteen discs. The TDI was fitted with a type N probe and set to reciprocate at a frequency of 2Hz.

Histology and microscopy

After testing, excised disc samples were embedded in paraffin, sectioned in the sagittal plane at six micrometers and stained with Safranin-O. The sections were imaged (Eclipse E800, Nikon, Melville, NY) under bright field to examine tissue structure, and under polarized light to assess collagen birefringence at 20×. For each captured slide image the grading was

oriented for maximum extinction of nonbirefringent light. Collagen birefringence under polarized light is an established method for assessing the fibril structure of soft tissues.^{1, 13}

Results

Annular stiffness, determined for each disc by averaging the slope parameter from ten cycles measured at a depth of 10% of disc diameter, ranged from 687 to 5646 N/m. Within the same spine the average stiffness parameter varied more than 400% between levels within the same lumbar segment. Large variability was also observed in nucleus mechanical parameters with average stiffness parameter ranging from 22 to 470 N/m. No significant correlation was present between average stiffness and Pfirrmann score ($p = 0.51$). In fact, no significant correlations existed between Pfirrmann score and any paired local mechanical data, including strain energy absorption values (Figure 2; all p 's > 0.50).

Results from comparative polarized light microscopy indicated a strong qualitative correlation between dense, well-defined striations in the annulus and high local stiffness. Specimens with less well-defined striations and lower lamellar density tended to have a low average stiffness profile (Figure 3). Pfirrmann score did not qualitatively correlate with morphologic features observed via polarized or non-polarized light microscopy.

Discussion

We hypothesized that mechanical profiles of stiffness or energy dissipation from the TDI would correlate well with Pfirrmann grading; this was not the case. Several discs that appeared healthy according to MRI and Pfirrmann grade had relatively homogenous stiffness profiles and disorganized fibril structure. While some discs that appeared less healthy according to Pfirrmann score showed steeply graded modulus profiles and well-organized lamellar structures. Overall, data from the TDI appeared better correlated with collagen structure, as indicated by lamellar striations, than with the Pfirrmann grade.

Our results indicate that the Pfirrmann grade does not tend to account for structural derangements in the lamellae of the annulus fibrosus or related differences in mechanical properties. However, MRI methods are sensitive to biochemical indicators of health, particularly water content. Since, as pointed out in the introduction, degeneration can be considered is an agglomeration of structural and biochemical deficiencies, we anticipate that the mechanical data from the TDI may supplement biochemical indicators of health to give a broadened perspective on what it means to have a degenerate disc. Further, the TDI can help better determine a quantitative relationship between biochemical and mechanical changes associated with degeneration.

One shortcoming of these early results is that observations of fibril order via polarized light microscopy are qualitative. In order to gage structural order in the annulus we simply noted the relative clarity of striations under polarized light. It is possible to do more to quantify organization using a mean intercept length algorithm or utilize new methods using polarization modulated second harmonic microscopy in intervertebral discs.¹² Future work will need to more thoroughly quantify collagen structure.

Despite this near-term shortcoming, results indicate that probing through the disc with high spatial resolution may be a way to reliably gage differences in mechanical properties and correlate them with differences in microstructural features. This tool could provide valuable information about disc properties and help us quantitatively evaluate how mechanical properties relate to degeneration. Given the TDI probes' small size (23 gauge needle; 0.573 mm), there is potential to assess mechanical properties *in vivo*.¹⁶ However, from a research

standpoint the current focus of testing is on generating useful *ex vivo* results and viewing the device as a mechanical tester capable of unprecedented spacial resolution.

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Figure 1.

(A) transverse x-ray image of a cadaver lumbar motion segment L1-L2 with TDI test probe in the annulus, and in the nucleus (B); (C) representative hysteresis loop plotting force versus displacement data in the annulus, and in the nucleus (D); the shaded area within the loop quantifies the strain energy dissipation and the least squares fitted line slope quantifies local modulus. (E) average slope and energy dissipation profiles for the disc are shown.

Figure 2. Average least squares slope and energy dissipation parameters given according to Pfirrmann grade. (n = 3,5,4,1 for grades I, II, III and IV, respectively)

Figure 3.

Sagittal polarized light images of Safranin-O stained sections highlight differing striated lamellar structure in relatively low stiffness profile annulus from L1-L2 spine 1011 (left) and relatively high stiffness profile annulus from L1-L2 spine 1201 (right). This contrast underscores an emerging pattern in this preliminary data.