Mechanical profiling of intervertebral discs
+1,2 Schultz, D S; 2Rodriguez, A G; 3Hansma, P K; 2Lotz, J C
+1University of California, Berkeley, CA  + 2University of California, San Francisco, CA  3University of California, Santa Barbara, CA
Senior author lotzj@orthosurg.ucsf.edu

ABSTRACT INTRODUCTION:
Linking degenerative tissue features to alterations in mechanical properties is central to understanding disc disease mechanisms. Yet, because not all degenerated discs are painful, it is critically important to identify those aspects of structural derangement that correlate with symptoms. By definition, therefore, this requires techniques that can be administered in vivo and provide the clinician with spatially-robust measures of disc material properties.

The Tissue Diagnostic Instrument (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 (Figure 1). The TDI was modified from the Bone Diagnostic Instrument (Hansma, P.K. et. al., 2008) so as to measure soft tissue mechanical properties subcutaneously at a spatial resolution on the order of 1 mm. We hypothesized that the TDI could detect changes in the viscoelastic properties across a disc; effectively generating a mechanical profile with high spatial resolution. It was further hypothesized that features of these mechanical profiles would correlate with Pfirrmann morphologic grading based on MRI and with histologic observations.

The TDI generates force versus displacement hysteresis curves (Figure 2). The slope of the force vs. displacement hysteresis loop (specifically, the slope of a least squares fitted line through the data) is an indication of local tissue modulus in the 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. This measurement offers a simple way of resolving the local elastic and viscous components of the tissue.

METHODS:
Magnetic resonance imaging: Four cadaveric human lumbar spines ranging in age from 32-72 were harvested and frozen (-20°C). 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. (Pfirrmann, C.W. et. al. 2001)

TDI mechanical testing: For each of the 13 discs, the probe was inserted in the medial-lateral direction along the disc mid-height to depths of 10, 20, 30, 40 and 50% (Figure 2), and the TDI set to reciprocate at a frequency of 2Hz.

Histology and microscopy: After testing, disc tissue samples were embedded in paraffin, sectioned in the sagittal plane at 6 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 20X. Collagen birefringence under polarized light is an established method for assessing the fibril structure of soft tissues.

RESULTS

Figure 2: (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) histogram comparing the average least squares slope in the annulus versus that in the nucleus. (F) comparison of average energy absorbed.

Average local annular moduli ranged from 687 to 5646 N/m, roughly one order of magnitude difference. Within the same spine, local annular moduli varied more than 400% between levels. Large variability was also observed in the nucleus with average local nucleus pulposus moduli measured at 50% of disc diameter ranging from 22 to 470 N/m. No significant correlation was apparent between average local annular moduli and Pfirrmann score (p=0.51). In fact, no significant correlations existed between Pfirrmann score and any pair of local mechanical data, including strain energy absorption values (p>0.50). By contrast, there was strong qualitative correlation between dense, well-defined striations in the annulus observed by polarized light microscopy and high local moduli.

DISCUSSION:
We hypothesized that mechanical profiles of local moduli 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 inhomogeneous modulus 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 was better correlated with collagen shape than the MRI Pfirrmann grade. This suggests that the TDI provides a mechanism to distinguish mechanically-relevant changes in tissue features that are unappreciated on traditional MRI imaging.

Given the TDI probes’ small size (23 gauge needle; Ø 0.573 mm), these mechanical features can be assessed in patients. Our results suggest that minimally invasive 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. However, clinical relevance and diagnostic utility will depend on in vivo testing. (Funded by: NIH grant RO1 GM 65554, NIH grant RO1 AR 049770)