Comparing Strip Biaxial vs Equibiaxial Loading: The Importance of Biaxial Testing Annulus Fibrosus Lamellae

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INTRODUCTION
About two thirds of adults in the United States experience low back pain at some point in their lives and low back pain continues to be the most common type of pain reported by adults [1]. Causes are generally unknown, although one theory attributes low back pain originating with intervertebral disc degeneration. To understand low back pain better, much research has focused on fundamental understanding of the disc. Physiologically analogous material properties may support improved modeling and understanding of the disease progression. Unfortunately, to date, few material property data, collected akin to the in vivo environment, exist. Spinal bending places the anterior annulus fibrosus (AF) in equibiaxial loading. However, with the exception of Bass et al. [2] which used three samples, discrete strain tracking, and a low strain protocol, human cadaveric lamellae biaxial testing has remained unexplored. Thus the objective of this research was to compare the suitability of strip biaxial and equibiaxial loading of cadaveric single AF lamellae to provide mechanical properties of the tissue and support advanced tissue modeling.

METHODS
Four thawed, freshly frozen human cadaveric L1-L2 segments were cut transversely with a bone saw to extract the disc with vertebral attachments above and below the disc. The disc was cut parallel to the frontal plane to excise the most anterior portion of the disc. A rotary tool was used to cut the vertebral attachments along the sagittal plane. The samples were dissected to single lamellae. The final sample shape is shown in Figure 1a.

Prior to biaxial testing, cyanoacrylate glue was used to attach sandpaper to each arm. The tissue was speckled with Verhoeff stain for strain tracking, placed in the grips of each load cell, with the axial grips attached to vertebrae and circumferential grips to the lamellae, and immersed in a saline bath as seen in Figure 1b. A pre-load of 0.1N was applied to each arm, and a three-step protocol was performed. The protocol cycled through one 7% equibiaxial strain and two orthogonal 7% strip biaxial strains. The strip biaxial test is a uniaxial extension test holding the orthogonal axis fixed. The protocol was performed on anInstron-Sacks biaxial tester.

Vector plots superimposed with rotation angle contour plots showing the 1st and 2nd principal strains vectors and rotation angles were calculated by passing the images through a custom code performing continuous image correlation strain tracking. The 1st principal strain is plotted orthogonal with the 2nd.

Paired t-tests were performed to compare the average force calculated at the peak strain in the two directions under the loading conditions.

RESULTS
Strip biaxial and equibiaxial force-stretch and principal strain vector plots from the same loading condition in the protocol are shown together (Figures 2-4). The vector plots show the tracked surface of a representative sample in the current configuration and the corresponding loading condition. In the reference configuration, the representative sample had a fiber orientation of roughly 60° from horizontal. Plots showing the forces normalized by the arm length versus stretch are shown as an average of four data sets with 95% confidence intervals. The force/stretch data in Figure 3a show the most pronounced curves for the two directions of the three loading conditions. Each rotation angle plot displays distinct areas of motion toward the displacing arms, and the equibiaxial principal strain vectors appear larger than in the other loading conditions.

Using a paired t-test, the final forces calculated in each protocol in the axial and circumferential directions were compared. Comparing the circumferential data from the equibiaxial test and circumferential strip biaxial test produced a significant p value (p=0.01). Other comparisons produced trending data. Comparing the axial data from the equibiaxial test and the axial strip biaxial test produced a p value that showed a trend (p=0.10). In addition, comparing the axial to the circumferential direction, the equibiaxial test p value showed a trend (p=0.14) while the strip biaxial tests p value showed a weaker trend (p=0.22).

DISCUSSION
Statistical analysis shows a moderate difference in the loading between the two directions in the equibiaxial test, while there is little difference between the two directions in the respective strip biaxial tests. In spite of fiber alignment in the tissue, no statistically significant mechanical anisotropy was observed. However, the axial data from the equibiaxial and axial strip biaxial test show a trend towards statistical significance, and the circumferential data from the equibiaxial and circumferential strip biaxial test show statistical significance. Also, the principal strain vectors demonstrate the tissue to be stiffer in the equibiaxial than the strip biaxial tests, so the respective strip biaxial tests capture different mechanical properties of the tissue than the equibiaxial test. Therefore, comparing strip biaxial loading to equibiaxial loading demonstrates the incomplete nature of the material properties measured under strip biaxial loading alone.

These data constitute a step towards characterizing the material properties of AF lamellae and support use of biaxial testing for characterization. The statistical strength of these data is limited due to the sample size; however given the observed trends, future work with a larger sample size is justified.

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REFERENCES