Physiological Biaxial Boundary Conditions Affects Stress-Stretch Behavior of the Annulus Fibrosus

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INTRODUCTION:

The annulus fibrosus (AF) experiences complex multi-directional loads *in vivo* (Fig 1A). Uniaxial tensile properties of the AF have been widely studied; [1-3] however, the freely contracting edges required in uniaxial tensile tests do not match the *in situ* AF boundary conditions which are constrained by the hoop structure and insertion into the vertebral body. Uniaxial tensile tests in the disc axial direction do not engage the fibers, resulting in a very low stress-strain response [4, 5] and, therefore, spurious material properties. Biaxial tension has been widely investigated for cardiovascular fiber-reinforced tissues, [6, 7] but has limited application for the AF. [4] **Therefore, the objective of this study was to evaluate the biaxial tensile properties of the AF in the circumferential-axial plane.** We hypothesize that the apparent modulus will increase in both orientations, as the stress on the transverse direction is increased.

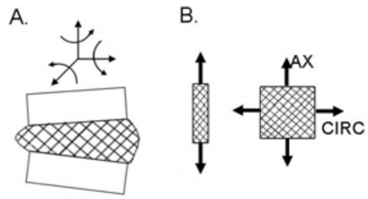

Fig 1: (A) Schematic showing *in situ* AF boundary conditions and multi-directional loading, (B) Uniaxial and biaxial sample geometry and loading

METHODS:

Planar square samples (n=6, 9 x 9 mm, 2 mm thick) were prepared from bovine caudal discs, with sides aligned along the circumferential (CIRC) and axial (AX) directions in the anterior AF (Fig 1B). Biaxial testing was performed on a custom built instrument [7] under three CIRC:AX strain ratios, including AX-fixed, 2:1 and 1:1. A preload of 0.5N and 0.25N was applied in the CIRC and AX directions, respectively, followed by a constant strain rate of 0.01%/s to a maximum strain of 6% or a stress limit of 1MPa. Preliminary studies determined the necessary recovery time between loading (1hr for AX-fixed and 2 hrs for 1:1) and confirmed no damage occurred. Following biaxial testing, the samples were cut to 7 mm long, 7 mm wide and tested in uniaxial tension along the CIRC or AX direction. Two-dimensional Lagrangian strain was calculated from images of four markers glued to the sample surface. A bi-linear curvefit to the stress-strain response was used to calculate the apparent toe- and linear-region moduli and the transition strain between these two regions. A one-way ANOVA with repeated measures was performed to compare uniaxial and biaxial properties, and a Bonferroni post hoc test was performed once significance was found (GraphPad, Inc. 4.03). Significance was set at $p < 0.05$.

RESULTS:

The uniaxial and biaxial stress-strain behavior was nonlinear for both the CIRC and AX orientations (Fig 2A & B); although the uniaxial transition to the linear region for the AX orientation occurred at high strains (15%, not shown). The transition strain from the toe-region to linear-region was lower in biaxial loading compared to uniaxial loading in both the CIRC and AX direction. In the CIRC direction, the biaxial transition strain (0.029 ± 0.010) was 3-fold lower than the uniaxial (0.08 ± 0.03; $p < 0.05$). In the AX direction, the biaxial transition strain (0.015 ± 0.006) was 10X lower than in uniaxial loading (0.15 ± 0.04, $p < 0.05$).

In the CIRC direction, the biaxial apparent toe-region modulus was 6-12X higher than the uniaxial (Fig 2C, $p < 0.05$ for 2:1 & 1:1 compared to uniaxial). In the AX direction, the biaxial toe-region apparent modulus was 20-30X higher than the uniaxial toe-region modulus (0.56 ± 0.26 MPa, Fig 2D, $p < 0.01$). Similar behavior was observed for the linear-region moduli. In the CIRC direction, the biaxial apparent linear-region modulus was 2-5X higher than the uniaxial modulus (Fig 2E, $p < 0.01$ for 2:1 & 1:1 compared to uniaxial). In the AX direction, the biaxial apparent linear-region modulus was 9-12X higher than the uniaxial modulus (2.62 ± 2.26 MPa, Fig 2F, $p < 0.01$). In most cases, the biaxial apparent moduli were significantly different between the three applied strain ratios, but to much lesser magnitude than uniaxial loading (Fig 2C-F).

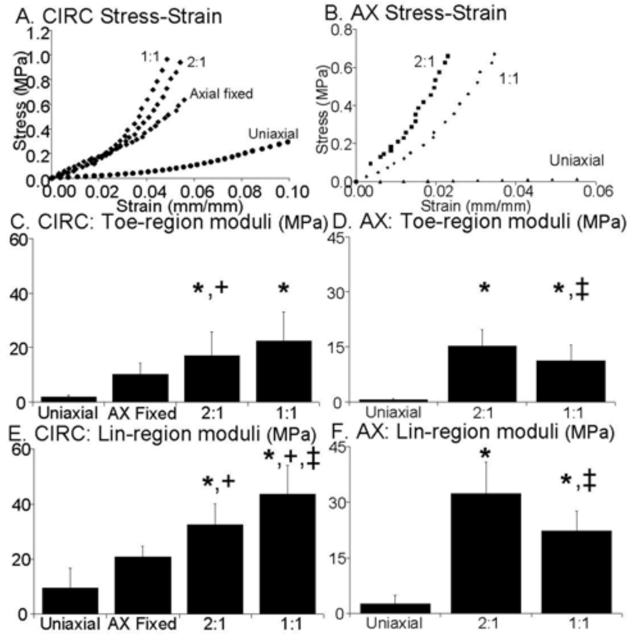

Fig 2: (A & B) Representative stress-strain behavior, (C & D) toe-region, (E & F) and linear-region moduli of the CIRC and AX directions, respectively. Symbols denote significance with respect to uniaxial (*), AX-fixed (+) and the 2:1 (‡) loading conditions.

DISCUSSION:

This study compared biaxial mechanics of the annulus fibrosus to uniaxial mechanical properties, with samples oriented along the circumferential and axial directions. The stress-strain behavior observed during uniaxal testing was consistent with previous studies in human AF. [1-5] Biaxial testing had a 2- to 12-fold higher apparent linear-region modulus compared to uniaxial testing. The larger apparent modulus is due in part to the applied constraint. However, the AX direction was most markedly affected (Fig 2B, D, & F) and this difference is likely due to more than the boundary constraint. The low stress and exaggerated toe-region in uniaxial loading (Fig 2B) suggests that the fibers are not engaged for this sample geometry, and this has been confirmed by fiber-reinforced constitutive modeling. [5] In biaxial tensile loading, where the fibers remain stretched, the AX stresses are much higher and of a similar magnitude to the CIRC stresses. Therefore, in the AX direction, the uniaxial material properties do not represent true AF functional mechanics and should not be used for modeling or tissue engineering benchmarks. In addition, the AF *in situ* geometry does not permit freely contracting edges, so this loading is less physiologically relevant than biaxial loading. Finally, biaxial testing loads the sample through a larger domain of strain configurations, as are experienced *in situ*, while uniaxial tests represent a single path within that domain. It has been shown for cardiac tissue that model parameters determined from biaxial experiments can be used to predict uniaxial behavior, but that uniaxial tests does not predict biaxial behavior. [8] Thus, uniaxial mechanics alone is not sufficient to quantify AF function. Accurate modeling of the tissue is important to design the optimal replacement device or to evaluate the effect of various surgical treatments. These models require input of accurate material properties, which are best quantified for the AF through biaxial testing.

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