Biaxial Mechanics are Inhomogeneous and Altered with Degeneration in the Human Annulus Fibrosus

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Introduction:

The mechanical function of the annulus fibrosus (AF) has been widely evaluated in uniaxial tension, demonstrating that the AF is highly anisotropic and the inner AF circumferential modulus is significantly lower than the outer AF [1]. Biaxial tension better reflects the loading environment experienced by the AF. We and others have shown that AF stiffness is higher in biaxial compared to uniaxial tension [2,3]. Importantly, in the axial direction of the spine (Fig 1), the measured uniaxial tensile properties are spurious due to transverse contraction (Poisson's ratio effect) that prevents fiber stretch in this orientation [4,5]. Biaxial loading constrains the transverse direction to obtain physiologically relevant loading; however, it requires a constitutive model to appropriately calculate material properties and to compare experimental data across groups. We have established an AF constitutive model based on strain energy of microstructural components, such as the matrix, fibers and fiber-matrix interactions [6]. The AF material properties measured in biaxial loading are unknown. Therefore, the objective of this study was to use biaxial tensile tests and constitutive modeling to evaluate the material properties of the inner and outer AF in the circumferential (CIRC), axial, and radial directions for non-degenerate and degenerate discs.

Methods:

Planar square samples (9 x 9 mm, 2 mm thick) were prepared from human lumbar discs (L3-L4 and/or L4-L5), with sides aligned along the CIRC-axial and radial-axial directions in the outer (n = 16) and inner AF (n = 9; Fig 1). $T_{1\rho}$ relaxation times were calculated as a measure of degeneration (age = 25–80 yrs, $T_{1\rho}$ time = 46–146ms) [7]. In the CIRC and axial directions a preload of 0.50N and 0.25N was applied in the outer and inner AF, respectively. A 0.25N preload was applied in the radial direction. A 10 cycle precondition was applied from 0 to 2%, followed by a constant strain at a rate of 0.01%/s. CIRC-axial samples were tested in equibiaxial loading, and radial-axial samples were testing with the axial direction held fixed at zero strain. 2D Lagrangian strains were calculated from four markers on the sample surface.

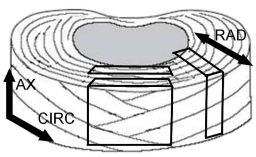

Fig 1. Schematic of sample orientations and locations.

A nonlinear, anisotropic, hyperelastic constitutive model was applied to determine the material properties as previously described [6]. The strain energy of the tissue was described as a combination of extrafibrillar matrix (W_m), fibers (W_f) and normal fiber-matrix interaction (nFMI; W_{nFMI}).

$$W_m = c_1(I_1 - 3) + c_2(I_2 - 3) + c_3(I_3^{1/2} - 1)^2 - 2(c_1 + 2c_2)\ln I_3^{1/2}$$

$$W_f = \sum_{\alpha=4,6} \frac{c_4}{2c_5}(e^{c_5(I_\alpha - 1)^2} - 1)$$

$$W_{nFMI} = c_6\left[\left(\tfrac{1}{2}I_1^2 - I_1 - I_2 + \tfrac{1}{2}\right) + \tfrac{1}{2}\left(\tfrac{1}{2}(I_4^2 + I_6^2) - (I_5 + I_7) + 1\right]\right.,$$

where c_i parameters are the material properties and I_a's are the invariants of the deformation tensor. A least-squares solution was used to find the best-fit parameters, $c_{1\text{-}6}$. The relative stress contribution of each model component to the overall stress was determined for the linear-region defined as the midpoint between the transition strain and the end of the test [4]. A t-test was performed to compare the material properties (c_4-c_6) and the relative stress contribution of each component (i.e. matrix, fiber, and nFMI) between the inner and outer AF. A Pearson's correlation was performed to determine the effect of degeneration on the outer AF material properties (c_1-c_6). Significance was set at $p \leq 0.05$.

Results:

The stress-strain response in biaxial loading was nonlinear and the model described the behavior well in all directions ($R^2 > 0.98$; Fig 2). The matrix properties c_1-c_3 were 1.1 ± 0.8 MPa, -1.0 ± 0.8 MPa and 1.5 ± 1.7 MPa, respectively, and were not dependent on degeneration ($p \geq 0.2$). In the outer AF, the fiber properties, c_4 and c_5, were 2.0 ± 0.8 MPa and 131 ± 61, respectively, and the nFMI, c_6, was 2.2 ± 1.5. In the inner AF, the tissue experienced lower stresses and an elongated toe-region (Fig 2). All inner AF properties were less than the outer AF ($p \leq 0.01$; inner AF c_4 = 0.4 ± 0.5 MPa, c_5 = 64 ± 33, & c_6 = 0.7 ± 0.1 MPa). The

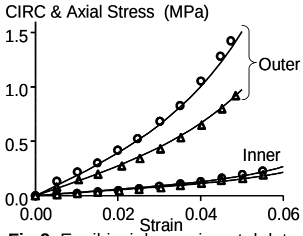

Fig 2. Equibiaxial experimental data with the model-fit (solid line) of the CIRC (circles) and axial (triangles) directions in the inner and outer AF.

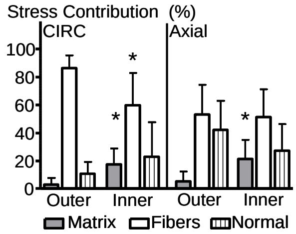

Fig 3. Stress contribution in CIRC and axial directions. * = p < 0.01 between the inner and outer AF.

transition strain used to determine the relative stress contribution in the linear-region was 0.02 ± 0.01 in the outer AF and 0.04 ± 0.01 in the inner AF. In the CIRC direction, the stress contribution in the outer AF was dominated by the fibers (86%) followed by the nFMI (10%) and the matrix (3%; Fig 3). The inner AF relative stress contribution from the fibers was significantly lower (60%) than the outer AF while the matrix contribution was significantly higher (17%; Fig 3). In the axial direction, the outer AF stress contribution was similarly influenced by both the fibers (53%) and nFMI (42%), followed by the matrix. The matrix contribution in the outer AF (5%) was significantly lower than the inner AF (21%, p<0.05), and no differences were observed in the fibers or nFMI (Fig 3). There was a significant correlation between the outer AF material properties and degeneration: the fiber property (c_5) and the nFMI property (c_6) both decreased with degeneration ($p \leq 0.05$; Fig 4). Material properties for nondegenerate ($T_{1\rho}$ = 150ms) and degenerate ($T_{1\rho}$ = 50ms) were used to observe the difference in the average stress-strain response (Fig 5).

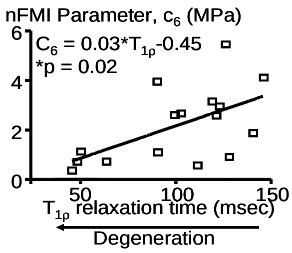

Fig 4. The nFMI property correlates with degeneration.

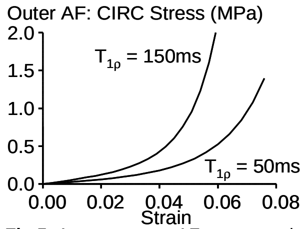

Fig 5. Average outer AF stress-strain response for nondegenerate (150ms) is higher than degenerate (50ms)

Discussion:

This was the first study to evaluate inner and outer human AF material properties and the effect of degeneration using biaxial testing. The inner AF was less stiff with an elongated toe-region, which is consistent with uniaxial tension [1]. The fiber and nFMI material properties were higher, and the relative stress contribution of the matrix was lower in the outer AF compared to the inner AF. These differences can be explained by the compositional variation, where the outer AF has more collagen type I, less collagen type II, and less glycosaminoglycan compared to inner AF [6]. The nFMI differences may also be related to other minor structural proteins, such as elastin.

Degenerative changes in the AF mechanical properties have been elusive in uniaxial tension [1,4,6]; however, biaxial tensile testing demonstrated differences with degeneration that were striking (Fig 5). These decreases in the fiber nonlinearity (c_5) and nFMI result in decreased stiffness and an elongated toe-region. The microstructural and compositional contributors to these changes are currently unknown and are the focus of future studies. Future work will also add samples to evaluate degeneration in the inner AF.

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Reference: [1] Acaroglu+ Spine: 20(24), 1995; [2] Bass+ Ann Biomed Eng: 32(9), 2004; [3] O'Connell+ 55th ORS 2009; [4] O'Connell+ J Biomech Eng *In Press* 2009; [5] Adams+ Eur Spine J: 2(4) 1993; [6] Guerin+ JOR: 25(4), 2007; [7] Johannessen+ Spine: 31(11), 2006; [8] Antoniou+ JClin Invest: 98(4), 1996