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Theoretical and Uniaxial Experimental Evaluation of Human Annulus Fibrosus Degeneration

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Abstract

Background—The highly organized structure and composition of the annulus fibrosus provides the tissue with mechanical behaviors that include anisotropy and nonlinearity. Mathematical models are necessary to interpret and elucidate the meaning of directly measured mechanical properties, to understand the structure-function relationships of the tissue components, namely the fibers and extracellular matrix. This study models the annulus fibrosus as a combination of strain energy functions describing the fibers, matrix, and their interactions. The objective was to quantify the behavior of both nondegenerate and degenerate annulus fibrosus tissue using uniaxial tensile experimental data.

Method of Approach—Mechanical testing was performed with samples oriented along the circumferential, axial, and radial directions.

Results—For samples oriented along the radial direction, the toe-region modulus was 2.7X stiffer with degeneration. However, no other differences in measured mechanical properties were observed with degeneration. The constitutive model fit well to samples oriented along the radial and circumferential directions ($R^2 = 0.97$). The fibers supported the highest proportion of stress for circumferential loading at 60%. There was a 70% decrease in the matrix contribution to stress from the toe- to the linear-region of both nondegenerate and degenerate tissue. The shear fiber-matrix interaction contribution increased 125% with degeneration in the linear-region. Samples oriented along the radial and axial direction behaved similarly under uniaxial tension (modulus = 0.32MPa versus 0.37MPa), suggesting that uniaxial testing in the axial direction is not appropriate for quantifying the mechanics of a fiber reinforcement in the annulus.

Conclusions—In conclusion, the structurally motivated nonlinear, anisotropic hyperelastic constitutive model help to further understand the effect of microstructural changes with degeneration, suggesting that a remodeling in the subcomponents (i.e. the collagen fibers and FMI) may minimize the overall change in tissue mechanics with degeneration.

Keywords

intervertebral disc; degeneration; tensile properties; nonlinearity; anisotropic hyperelastic model; constitutive modeling

INTRODUCTION

The intervertebral disc is comprised of complex components that provide the disc with nonlinear, anisotropic, and viscoelastic mechanical behaviors. These disc behaviors permit motion, transfer spinal loads, and absorb energy. The annulus fibrosus (AF) is a highly organized structure composed of concentric layers of collagen fibers oriented at $\pm 28\text{--}30^\circ$ to the horizontal plane and are embedded in a proteoglycan extracellular matrix.[1] The AF has a high tensile stiffness[2–4] and supports the large multi-directional loads encountered by the disc, such as tension, compression, shear, torsion, and bending. The fibrous structure and composition of the annulus provides the tissue with its mechanical behaviors including high stiffness and strength, anisotropy, and nonlinearity.[2, 3, 5–7] Biochemical changes with degeneration in the AF include a decrease in water and proteoglycan content and an increase in type I and II collagen.[8–11] The annulus becomes stiffer with degeneration under shear and confined compression; however, the tensile modulus of the AF tissue has not demonstrated significant changes with degeneration.[3, 5, 12, 13]

The layered structure and orientation of the collagen fibers in the extracellular matrix provides the tissue with its anisotropic and inhomogeneous mechanical behavior. Uncrimping of collagen fibers and fiber-matrix interactions are thought to contribute to the nonlinear behavior of the tissue.[14–16] The behavior of the tissue's fibers and matrix are generally accepted to contribute to tensile and compressive behaviors and have been used to describe the AF.[4, 6, 7, 17–20] However, other components, which are not as well understood, include minor collagens, small proteoglycans, and collagen crosslinks. While these components have been identified in the AF,[21–23] their contribution to the mechanical behavior of the AF is unknown.[3, 19, 20, 24] Since it is difficult to experimentally quantify the mechanics of these individual components, continuum models can be used to describe them.

Continuum models are useful to interpret and elucidate the meaning of experimental measurements made in mechanical tests. Based upon the classic work of Spencer,[25] the AF has been modeled as a fiber-induced anisotropic hyperelastic material (e.g., refs [4, 6, 7, 18, 20, 24, 26, 27]), using the principle invariants of the Green deformation tensor and structural tensors representing the collagen fiber populations. Contributions of interactions between the proteoglycans and collagen fibers can be incorporated into models through fiber-matrix interactions, making these models potentially useful for studying the effects of degeneration.[4, 7, 20] Wagner and Lotz recently used chemical glycation to crosslink human AF and, after adjusting the stress-strain response for the softening effects of soaking the tissue, showed an increase in axial direction stiffness which could be modeled by including fiber-matrix interactions.[24] Notably, previous AF continuum models have not been applied to experimental data from both nondegenerate and degenerate tissue. Constitutive modeling applied to both nondegenerate and degenerate AF may elucidate microstructural changes with degeneration, will be useful for finite element models,[6] and provide targets for disc treatments, such as tissue engineered constructs.[28]

A previous study in our laboratory derived a structurally motivated nonlinear, anisotropic, hyperelastic model, which described nondegenerate AF tissue using strain energy equations for the collagen fibers, extracellular matrix, and fiber-matrix interactions (FMI) fit to tensile experimental data oriented along the radial, axial and circumferential directions.[7] This study described fiber-matrix interaction terms both shear and normal to the fiber direction. The FMI term parallel (shear) to the fibers was described as a nonlinear function, while the normal FMI was linear. This study concluded that the interaction terms have a large contribution to the AF stress in uniaxial tension. However, the study was limited by the contraction (compression) of the fibers in the axial direction and was only used to describe

nondegenerate AF tissue. The current study modified the established model and described the behavior of both nondegenerate and degenerate AF tissue, using uniaxial tensile experimental data. The current model was modified by excluding the normal FMI term and changing the shear FMI term to a rotation of the fiber population rather than a rotation of the measured strains (Mohr's circle).[7, 20] Furthermore, the model was used to quantify the stress contribution by each component and evaluate the changes with degeneration. Few degenerative changes are observed in the mechanical properties of the AF at the tissue level, [3, 5] despite significant changes in the compositional and structural level.[8, 9] The objective of this study was to determine the effect of degeneration at the microstructural level, using a continuum model, to provide insight to the changes made with degeneration that help to preserve the mechanical behavior of the tissue on the macrostructural level. We hypothesized in this study that fiber-matrix intralamellar interactions significantly contribute to both nondegenerate and degenerate tissue, with a significant increase in the fiber-matrix contribution with degeneration.

METHODS AND MATERIALS

Mechanical Testing

Intervertebral discs from the L3-L4 and the L4-L5 levels were dissected from seven human cadaveric lumbar spines, as previously described.[3] Three separate reviewers graded the samples using the Thompson scale, modified for axial sections, [29] with an average grade below 2.5 considered to be nondegenerate ($n = 7$, age 36–53 years old, average grade = 2.2 ± 0.3)[3] and greater than 2.5 as degenerate ($n = 6$, age 53–80 years old, average grade = 3.9 ± 0.7). Samples were prepared from the outer anterior AF that did not contain any annular defects, oriented along the circumferential, axial and radial directions (length $\times$ width $\times$ thickness: $15.8 \times 2.8 \times 1.9$ mm, $11.1 \times 2.3 \times 1.5$ mm and $14.2 \times 2.5 \times 1.8$ mm, respectively; Figure 1). Samples were preloaded for 10 minutes at 0.1N in the circumferential and axial directions and 0.05N in the radial direction. A 20 cycle precondition was applied from 0–2% strain; then the samples were stretched in a uniaxial quasi-static ramp test,[3] with optical images acquired for quantification of two-dimensional Lagrangian tissue strain (**E**) (Vic2D, Correlated Solutions, Inc.). Since the previously published data (for the nondegenerate samples) used a different custom-written program to calculate strains, the strains were re-calculated for this study using the Vic2D software. The Lagrangian stress (**S**) was calculated as force divided by the undeformed cross sectional area.

The components of the right Cauchy-Green deformation tensor (**C**) were calculated from measured Lagrangian strain using $\mathbf{C} = 2\mathbf{E} + \mathbf{I}$, where **I** is the identity matrix ($\mathbf{C} = \mathbf{F}^T\mathbf{F}$, where **F** is the deformation gradient). Multidimensional datasets were constructed using the stress and strain components and Poisson's ratio data from matched circumferential, axial and radial samples, as previously described.[7] The toe- and linear-region modulus and the Poisson's ratio were calculated from the uniaxial tensile tests.[2, 3] The toe- and linear-region moduli were calculated using a bi-linear regression algorithm, with the linear fits extended to determine the intersection point, which was defined as the transition strain.

Constitutive Modeling

A structurally motivated anisotropic hyperelastic model for finite deformations was formulated based upon the work of Spencer[25] and as previously published.[7] The strain energy function was described as a combination of each structural component using the integrity basis of invariants, formed from the deformation tensor **C** and the unit fiber direction vectors **a** and **b**:

$$\begin{aligned}
I_1 &= \text{tr} C & I_4 &= a \cdot C \cdot a & I_6 &= b \cdot C \cdot b \\
I_2 &= 1/2[(\text{tr} C)^2 - \text{tr} C^2] & I_5 &= a \cdot C^2 \cdot a & I_7 &= b \cdot C^2 \cdot b \\
I_3 &= \det C
\end{aligned} \tag{1}$$

The invariants involving fiber stretch ($I_4 - I_7$) were restricted to be greater than or equal to 1.0; therefore, assuming that the collagen fibers buckle under compression. The individual strain energy equations were required to be positive definite, $W_i \geq 0$. The annulus fibrosus tissue was modeled with components describing the matrix (W_m), fibers (W_f) and their interactions (W_{shearFMI}). The c_i 's in the following equations represent the model parameters, which were determined by fitting the strain energy functions to experimental data as described below.

The matrix strain energy (W_m) was described as a coupled compressive Mooney-Rivlin material, which is a function of I_1 , I_2 , $J = \det(\mathbf{F})$, and model parameters c_1 , c_2 and c_3 which have units of MPa.[7, 30]

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

The fiber strain energy (W_f) was described as the sum of two exponential equations dependent on the fiber stretch invariants (I_4 & I_6), as used in previous models for the AF.[6, 7, 31] The model parameter c_4 has units of MPa and c_5 is unitless.

$$W_f = \sum_{\alpha=4,6} \frac{c_4}{2c_5} \left(e^{c_5(I_\alpha - 1)^2} - 1 \right) \tag{3}$$

The shear fiber-matrix interaction (FMI) represents the energy transfer parallel to the fibers. [4, 7] The fiber-matrix interaction terms used previously in our model was derived from the two-dimensional rotation of Mohr's circle, resulting in both a shear and a normal interaction term.[4, 7, 24] The current model improves upon the description of the shear interaction component by describing the bond between the fiber and matrix as a three-dimensional geometric rotation of the fiber population, as recently proposed by Peng et al., [20] rather than describing the shear FMI using a two-dimensional rotation of strains using Mohr's circle.[4, 7, 24] The strain energy function for the shear FMI term is derived using Nanson's relation for the normal vector and by describing the rotation in terms of the basis of invariants. The strain energy equation presented here was simplified from the equation proposed by Peng et al., by fitting the shear term to a single parameter, c_6 , rather than to a function dependent on the fiber stretch invariant (I_4). This choice was supported based on preliminary model fits in which the experimental nonlinearity was well-described by the simplified function. The shear FMI term is described as a nonlinear function, since it is thought that the shear increases as the fibers become engaged during the linear region of a stress-strain curve. Furthermore, experimental studies of collagenous tissues in shear have observed a nonlinear stress-strain behavior.[32] The model parameter c_6 has units of MPa.

$$W_{\text{shearFMI}} = \sum_{\alpha=4,6} \left(c_6 \left(\frac{I_\alpha}{I_3} (I_{\alpha+1} - I_1 I_\alpha + I_2) - 1 \right)^2 \right), \tag{4}$$

where $\alpha+1$ represent I_5 and I_7 for α equals 4 and 6, respectively.

The strain energy equations were differentiated with respect to the deformation tensor to

obtain the symmetrical 2nd Piola-Kirchhoff stress tensor: $S = 2 \frac{\partial W}{\partial C}$. The model was fit to the

uniaxial tensile experimental data using a least-squares solution to determine the model parameters, as previously described.[3] Briefly, the tissue oriented along the radial direction was assumed to be a homogeneous structure only consisting of extrafibrillar matrix. Although there are collagen fibers in the plane perpendicular to the loading direction, they would only experience compression under this loading condition, which is not permitted in this model ($I_4 - I_7 = 1.0$). Therefore, the radial experimental data were fit first using the stress-stretch equation derived from W_m , to determine c_1 , c_2 and c_3 . Traction-free boundary conditions were applied using only the isotropic matrix term. Then, the stress-stretch equation derived from the full strain energy equation ($W = W_m + W_f + W_{\text{shearFMI}}$) was fit to the axial and circumferential data using the matrix constants to determine c_4 , c_5 and c_6 . The contribution of each component of the model (i.e. matrix, fibers or shear FMI), to the overall stress was determined for the toe- and linear-regions of the stress-stretch curve.

Preliminary studies varied the initial parameters by two orders of magnitude to ensure that the model was robust to changes in the initial parameters used for the six parameters. A correlation coefficient (R^2) value was calculated to provide a measure of association of the model fit to the experimental data. A Bland-Altman analysis[33] was used to determine bias and standard deviation of residuals for the separate orientations, to provide a measure of agreement between the model and experimental data.[34] A sensitivity analysis was

performed for each model parameter and was derived as $X^+(c_j) = \frac{c_{jo}}{S_{\max}} \frac{\partial S}{\partial c_j}$ where c_j is the j th constant being estimated, c_{jo} is a nominal value for that constant, and $S_{\max}$ is the maximum experimental 2nd Piola-Kirchhoff stress value of the function of interest.[35–37] The sensitivity coefficients for the stress-strain functions were evaluated at the midpoint of the loading direction deformation vector. Values above 0.1 indicated that the equation was sensitive to changes in that constant.

An unpaired Student's t-test was performed to compare nondegenerate and degenerate mechanical properties, model parameters and stress contribution. Pearson correlation coefficients were calculated to determine whether any inter-relationships existed between model parameters. The stress contribution from the toe- and linear-regions of the circumferential direction was compared using a paired t-test. Significance was set at $p = 0.05$.

RESULTS

Experimental Results

The radial direction stress-stretch behavior of the nondegenerate tissue was nonlinear, while the degenerate tissue was linear (Figure 2). The toe- and linear- region moduli, the transition strains and the Poisson's ratios are presented in Table 1. Comparing the radial modulus of samples over similar strains (i.e. below ~11% transition strain), the degenerate modulus was approximately 2X greater than the nondegenerate modulus ($p < 0.01$), with no differences in modulus above the transition strain ($p = 0.29$). There was no effect of degeneration for Poisson's ratio ($p = 0.14$). Samples oriented along the axial direction exhibited a linear stress-stretch behavior for both nondegenerate and degenerate AF tissue (Figure 3; Table 1). Some samples which were tested beyond 20% tensile strain showed signs of nonlinearity, resulting in an increasingly stiffer modulus of the tissue. Therefore, only the linear region below 20% strain was analyzed for consistency across samples and to keep the analyzed stress-strain range physiologically relevant. No significant differences were observed with the axial modulus and Poisson's ratio; however, the modulus decreased approximately 25% with degeneration ($p = 0.14$). For samples oriented along the circumferential direction, the stress-stretch behavior was highly nonlinear for both nondegenerate and degenerate tissue

(Figure 4; Table 1). No significant differences were measured in mechanical properties with degeneration (Table 1, $p > 0.33$).

Model Results

The constitutive model used to describe the AF tissue oriented along the radial and circumferential directions had an excellent fit for both orientations ($R^2 = 0.97$). The average and standard deviation for the model parameters are provided in Table 2. The nonlinear and linear behavior observed in the radial direction was well-described by the Mooney-Rivlin model (matrix parameters: c_1 , c_2 , and c_3) as observed by the high R^2 value and the very low bias (Figure 2, Table 3). The Mooney-Rivlin matrix parameters were 2.5-5X greater with degeneration ($p = 0.04$).

Simultaneously fitting the strain energy equation to the axial and circumferential data did not model the behavior of the axial data well ($R^2 = 0.70$). The fiber stretch term (I_4 , I_6) was constrained to 1.0 as described in the Methods; however, the fiber stretch for axial tensile testing was less than 1.0. Thus, due to fiber compression, the fiber and shear FMI terms were zero for the axial direction and it was assumed that the fibers had buckled. Therefore, only the experimental data oriented along circumferential direction was used to determine the model parameters for the fiber (W_f) and the shear FMI (W_{shearFMI}) terms, c_4 , c_5 and c_6 . Prediction of the stress-strain behavior in the axial direction using only the matrix term did not fit the experimental data well for nondegenerate samples ($R^2 = 0.44$ for nondegenerate & $R^2 = 0.79$ for degenerate).

The strain energy equation fit well to the circumferential data as seen by the high correlation and low bias ($R^2 = 0.98$; Figure 4; Table 3). There was no effect of degeneration on the fiber terms, c_4 and c_5 ($p > 0.2$; Table 2). There was approximately a 5X increase in the c_6 shear FMI parameter with degeneration ($p < 0.04$, Table 2). The percent stress contribution in the circumferential direction was calculated using the stress from individual components divided by the overall stress calculated from the model. In the toe-region, the fiber stretch was responsible for the greatest proportion of stress (63%, $n = 13$), followed by the shear FMI (27%), and the matrix (10%) contributed the least (Figure 5). In the linear region of nondegenerate tissue, the fibers contributed the greatest proportion of the total stress (65%), followed by the shear interaction term (32%), and the matrix (3%) contributed the least (Figure 5B). In the linear-region of degenerate tissue, the average stress contribution by the shear interaction (57%) and the fiber term (39%) were of similar magnitude, with the matrix contributing little (4%). There was no significant difference in relative stress contribution between nondegenerate and degenerated AF (in either the toe- or linear-region; $p = 0.09$; Figure 5). In comparing the stress contribution between the toe- and linear-regions, there were no significant changes for the nondegenerate tissue ($p = 0.17$); however, for degenerate tissue, a decrease was observed in the fiber contribution from the toe- to the linear-region, and an increase in the shear FMI contribution ($p < 0.02$; Figure 5).

There was a strong negative correlation between the matrix parameters c_1 and c_2 ($r = -0.99$; $p < 0.0001$), and between c_2 and c_3 ($r = -0.83$; $p < 0.01$); there was a strong positive correlation between matrix parameters c_1 and c_3 ($r = 0.85$; $p < 0.01$). The FMI model parameter, c_6 , had a positive correlation with the matrix parameter c_3 ($r = 0.58$, $p = 0.04$). No other significant correlations were found among the other model parameters. The sensitivity analysis for each model parameter demonstrated that the model fit for samples oriented along the radial direction were highly sensitive to the three matrix parameters, as defined by a sensitivity value greater than 0.1 (eight of 13 samples were sensitive to changes in c_3 , while 13 and 12 of the samples were sensitive to changes in c_1 and c_2 , respectively; Table 3). The model fit to the experimental data along the circumferential direction was not sensitive to the matrix parameters, but was sensitive to changes in the FMI parameter ($n = 7$,

for the number of samples sensitive to the parameter) and the fiber parameter, c_4 ($n = 10$; Table 3).

DISCUSSION

The structurally motivated nonlinear, anisotropic hyperelastic constitutive model was able to detect changes with degeneration in AF mechanical function even though the uniaxial tensile tissue properties (i.e. toe- and linear-region modulus) were not significantly altered with degeneration, as shown here and in previously published studies (Table 1).[3, 5] The model detected significant differences in the matrix parameters ($c_1 - c_3$) and the shear FMI parameter (c_6), suggesting changes with degeneration at the microstructural level. The current model improved upon the previous model published by our laboratory by constraining the fibers from being loaded in compression ($I_4 - I_1 = 1$), and by using a description of the interaction term between the collagen fibers and the extrafibrillar matrix based on the rotation of the unit vector representing the collagen fibers.[20] A good association and agreement between the experimental data and the model was observed by the high goodness-of-fit and the low bias, for samples oriented along the circumferential and radial direction (Table 3).

Recent models have included a FMI term perpendicular to the collagen fibers;[7, 24] however, applying a model which included a normal FMI term to the experimental data resulted in minimal stress contribution ($< 1\%$; data not shown). This suggests that, in uniaxial tension, the contributions of normal FMI are minor, compared to shear interactions between the fibers and the extracellular matrix; therefore, it was excluded from the model in this study.

This study elucidated the relative role of the matrix, fibers, and shear FMI in both nondegenerate and degenerate AF tissue in uniaxial tension oriented along the circumferential direction. The shear FMI term contributed a large portion of the applied stress ($>25\%$), and increased from the toe- to the linear-region for degenerate tissue, indicating that it is an important contributor to the mechanical behavior of the AF.[3, 19, 20, 24] The fibers contributed to the largest proportion of the toe-region stress in nondegenerate and degenerate AF tissue ($>60\%$). While a similar behavior was observed for the linear-region of nondegenerate tissue, the shear FMI component had a greater contribution than the fibers in the linear region of degenerate tissue. The large increase in the shear FMI may be due to an increased number of collagen crosslinks or a decrease in the mechanical integrity of the collagen fibers with degeneration. The extrafibrillar matrix component was the lowest stress contributor and decreased from 10% in the toe-region to less than 5% in the linear-region. There were no degenerative differences in the stress contribution by individual energy components, which may be due to the high standard deviations (Figure 5). In the toe-region of both nondegenerate and degenerate tissue, the fibers and shear FMI terms together contributed 90% of the stress, suggesting that the fibers are crucial for circumferential uniaxial tensile loading, even when the collagen fibers are thought to be uncrimping at low strains in the toe-region. As the fibers become fully engaged from the toe- to the linear-region, the contribution of the matrix diminishes, as observed in our previous model.[3]

A correlation analysis performed on the model parameters showed that the matrix parameters have a strong correlation with one another, and the shear FMI parameter was moderately correlated with the matrix parameter c_3 . It is unlikely that the correlation between the shear FMI and the matrix parameter provides significant findings in describing the AF tissue, since the matrix parameters were fit to the radial data separately. A sensitivity analysis showed that the model was highly sensitive to changes in the matrix material parameters in the radial direction. Along the circumferential direction, the model was

sensitive to changes in the fiber and FMI model parameters and highly insensitive to the matrix parameters.[38] This suggests high confidence in estimating unique matrix parameters using the radial experimental data and in estimating FMI and fiber parameters using the circumferential experimental data.

The mechanical properties measured in nondegenerate samples oriented along the radial direction are comparable with values reported in the literature.[2, 39, 40] Degeneration significantly altered the stress-strain behavior observed in the radial direction. Comparing the modulus in the same strain region ($< 11\%$), degenerate tissue was observed to be approximately 3X stiffer than nondegenerate tissue, resulting in a 2.5-5X increase in the magnitude of the three matrix parameters (Figure 1; Table 2). Although uniaxial samples oriented along the radial direction were described as loading only extrafibrillar matrix, the observed differences with degeneration may suggest that there are other microstructural contributions involved, which were not included in the current model. The observed decrease in the nonlinear behavior may be due to a change in the elastin content in the AF, which has been observed to increase by 5% with degeneration.[41] Furthermore, biochemical degradation of elastin resulted in a significant decrease in the modulus of the AF tissue in the radial direction.[40] This suggests that an increase in the amount of elastin could contribute to the increased stiffness observed in the radial direction. Moreover, the interlamellar elastin fibers have been observed as linear fibers with less crimping than intralamellar elastin fibers;[22, 42] therefore, an increase in the interlamellar elastin fibers may cause the stress-strain behavior to become linear with degeneration.

The mechanical properties measured along the circumferential direction is similar to the values reported in the literature;[2, 5, 43, 44] however, in the current study, no differences were observed with degeneration (i.e. Poisson's ratio and modulus). Aging and degeneration are difficult to discriminate between and no differences between them were observed in this study. However, the objective of the current study was only to determine the effects of degeneration. The Poisson's ratio results are in contradiction to the results reported by Acaroglu et al., where a 60% decrease in the outer AF Poisson's ratio was observed with degeneration (from 1.2 to 0.5),[5] and is the only sub-failure AF tensile mechanical property shown to change with degeneration. However, it is difficult to directly compare the Acaroglu findings to the results reported in this study because degeneration was grouped differently and the strains were measured differently. As a result, the mean values for the Poisson's ratio were higher in this study, with a non-significant 15% decrease with degeneration (2.3 for nondegenerate and 1.9 for degenerate). The lack of significance between values reported in this study may be due, in part, to the high standard deviations that were approximately 50% of the mean in the experimental results, which is typical for human tissue.

The stress-strain behavior of samples oriented in the axial direction was linear for physiological levels of strain (less than 20%). Samples which were tested to higher strains ($> 20\%$) exhibited a nonlinear behavior, which may due to the fibers becoming engaged at higher strain. The linear modulus of samples oriented along the axial direction was not different from the radial samples ($p = 0.3$), which also suggests that the fibers are not engaged for samples in this loading configuration. The delayed engagement of the collagen fibers at high strains ($> 20\%$) may be due to the boundary conditions of freely contracting edges used for uniaxial testing, which does not represent physiological loading conditions. Furthermore, the fibers would be loaded at lower strains *in vivo* due to having a wider effective width.[45] Because the fiber stretch was less than 1.0 in the axial direction, and therefore not contributing, the axial direction data was not used to determine model parameters.

Previous anisotropic hyperelastic models have modeled the AF tissue by only using experimental data from nondegenerate tissue. This study applied a constitutive model to both nondegenerate and degenerate human AF tissue to evaluate the degenerative effects on the subcomponents, such as the collagen fibers, extracellular matrix and the interactions between the fibers and matrix. Experimentally, the mechanical properties of the tissue changed little with degeneration, despite the tremendous degenerative changes that occur in the tissue composition and structure. Therefore, mathematical models are important to investigate how microstructural changes impact tissue function. The current model described the tissue as a combination of extracellular matrix, fibers and intralaminar shear fiber-matrix interactions; however, there are other microstructures that are currently not included in the model, such as collagen crosslinks and interlamellae interactions, and are the topic of future work. The results presented in this study help to further understand the effect of microstructural changes to the macrostructural level of the tissue, suggesting that the changes in the subcomponents (i.e. collagen fibers and FMI) of the tissue may change with degeneration to minimize the overall effects on mechanical function of the bulk material. Furthermore, the results from this model can be used to improve the accuracy of finite element models of the intervertebral disc, which provides information for optimizing the design of spinal implants and biological implants and will help to advance the knowledge of the degenerative process.

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Figure 1.
Schematic showing the orientation of axial, circumferential and radial samples taken from each disc.

Figure 2.

Representative experimental data (symbols) and model fit (solid line) to a nondegenerate and degenerate sample oriented along the radial direction. The nondegenerate tissue exhibited a nonlinear response, while the degenerate tissue exhibited a stiffer linear response. The constitutive model fit the experimental data well (solid line, $R^2 = 0.97$).

Figure 3. Representative experimental data from a nondegenerate sample (circles) and a degenerate sample (triangles) oriented along the axial direction.

Figure 4. Representative nondegenerate sample oriented along the circumferential direction with experimental data shown by the solid black circles and the model fit shown by the solid black line.

Figure 5.

Average stress contribution of the matrix (black), fibers (white), and shear fiber-matrix interactions (diagonal pattern) for A) the toe-region and B) linear-region of nondegenerate and degenerate tissue oriented along the circumferential direction. Error bars represent the standard deviation, * denotes significance with respect to the toe-region stress contribution ($p < 0.05$).

Table 1

Toe- and linear-region modulus, transition strain (E_{tr}), and Poisson's ratio for samples oriented along the radial, axial and circumferential directions for nondegenerate ($n = 7$), degenerate ($n = 6$) and pooled data (when applicable).

	Radial			Axial			Circumferential			
	Toe Modulus (MPa)	Linear Modulus (MPa)	E_{tr} strain (mm/mm)	Poisson's Ratio	Linear Modulus (MPa)	Poisson's Ratio	Toe Modulus (MPa)	Linear Modulus (MPa)	E_{tr} strain (mm/mm)	Poisson's Ratio
Nondegenerate	0.14 (0.05)	0.29 (0.08)	0.11 (0.04)	0.79 (0.42)	0.42 (0.11)	0.61 (0.35)	2.70 (2.33)	20.9 (13.5)	0.09 (0.06)	2.27 (0.87)
Degenerate	0.30 (0.09)	0.35 (0.13)	0.10 ^{**}	0.46 (0.31)	0.32 (0.11)	0.61 (0.28)	4.20 (2.99)	22.9 (10.9)	0.06 (0.02)	1.88 (1.01)
Pooled	0.21 (0.11)	0.32 (0.10)	N/A	0.63 (0.40)	0.37 (0.12)	0.61 (0.31)	3.39 (2.66)	21.8 (11.9)	0.08 (0.05)	2.09 (0.92)
p-value	<0.01 [*]	0.29	N/A	0.14	0.14	0.98	0.33	0.77	0.36	0.48

The reported p-values in the table are results of a Student's t-test comparing nondegenerate and degenerate AF, with the asterisk (*) denoting significant differences ($p < 0.05$).

^{**} in the radial data, denotes that only one degenerate sample had a nonlinear stress-strain behavior.

Table 2

Model parameters determined for both nondegenerate and degenerate tissue. Average (standard deviation).

	Matrix			Fibers		FMI
	c ₁ [*] (MPa)	c ₂ [*] (MPa)	c ₃ [*] (MPa)	c ₄ (MPa)	c ₅	c ₆ [*] (MPa)
Nondegenerate	0.11 (0.09)	-0.10 (0.09)	0.09 (0.11)	1.10 (1.87)	27.33 (33.87)	41.97 (52.70)
Degenerate	0.29 (0.14)	-0.24 (0.13)	0.43 (0.38)	1.04 (0.90)	6.39 (15.15)	200.4 (165.6)
p-value	0.02	0.04	0.04	0.9	0.2	0.04

The reported p-values in the table are results of a Student's t-test comparing nondegenerate and degenerate AF, with * denoting significance of $p < 0.05$.

Table 3

Goodness-of-fit (R^2), bias and standard deviation from the Bland-Altman analysis, and sensitivity results for nondegenerate and degenerate tissue oriented along the radial and circumferential directions. The sensitivity analysis for each model parameter is reported as the average and standard deviation of non-zero values.

	Radial						Circumferential									FMI
	Sensitivity - Matrix			Sensitivity - Matrix			Fibers			Sensitivity - Matrix			Fibers			
	R ²	Bias (MPa)	Resid SD (MPa)	c ₁	c ₂	c ₃	R ²	Bias (MPa)	Resid SD (MPa)	c ₁	c ₂	c ₃	c ₄	c ₅		
Nondegenerate	0.95	-0.0005	0.0013	0.90 (0.58)	0.66 (0.35)	0.28 (0.31)	0.98	0.006	0.052	0.03 (0.02)	0.02 (0.02)	0.01 (0.01)	0.21 (0.14)	0.15 (0.20)	0.14 (0.16)	
Degenerate	0.96	0.0001	0.0008	1.71 (0.84)	1.71 (1.30)	0.60 (0.57)	0.99	0.013	0.079	0.05 (0.04)	0.05 (0.05)	0.02 (0.02)	0.16 (0.11)	0.01 (0.02)	0.17 (0.10)	