A Computational Model to Describe the Regional Interlamellar Shear of the Annulus Fibrosus

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Interlamellar shear may play an important role in the homeostasis and degeneration of the intervertebral disk. Accurately modeling the shear behavior of the interlamellar compartment would enhance the study of its mechanobiology. In this study, physical experiments were utilized to describe interlamellar shear and define a constitutive model, which was implemented into a finite element analysis. Ovine annulus fibrosus (AF) specimens from three locations within the intervertebral disk (lateral, outer anterior, and inner anterior) were subjected to in vitro mechanical shear testing. The local shear stress–stretch relationship was described for the lamellae and across the interlamellar layer of the AF. A hyperelastic constitutive model was defined for interlamellar and lamellar materials at each location tested. The constitutive models were incorporated into a finite element model of a block of AF, which modeled the interlamellar and lamellar layers using a continuum description. The global shear behavior of the AF was compared between the finite element model and physical experiments. The shear moduli at the initial and final regions of the stress–strain curve were greater within the lamellae than across the interlamellar layer. The difference between interlamellar and lamellar shear was greater at the outer anterior AF than at the inner anterior region. The finite element model was shown to accurately predict the global shear behavior of the AF. Future studies incorporating finite element analysis of the interlamellar compartment may be useful for predicting its physiological mechanical behavior to inform the study of its mechanobiology. [DOI: 10.1115/1.4027061]

Keywords: interlamellar, shear, annulus fibrosus, finite element, constitutive model

Introduction

Degenerative disk disease continues to be a prevalent health and socioeconomic problem [1,2]. Current surgical treatments that involve the use of implants predominantly seek to arrest the degeneration cascade and do not attempt to repair tissue damage. In contrast, the goal of most intervertebral disk tissue engineering approaches is to affect the biology such that tissue damage is arrested or reversed [3–5] wherein restoration of the mechanical function and homeostasis of the healthy native tissue is achieved. It is well accepted that the cellular response is a function of the local mechanical environment. Thus, in order to engineer viable biomimetic replacements or repair strategies of the intervertebral disk, the mechanobiology of the native AF tissue must be well characterized such that appropriate design and acceptance criteria can be developed.

Microstructurally, the AF is very complex and consists of multiple lamellae with alternating collagen fiber orientations imbedded in a proteoglycan-rich ground matrix. Between each layer is a collagen and elastin rich interlamellar compartment [6,7]. Morphologically distinct cells provide evidence that the interlamellar compartment may be biologically active and independent from its lamellar counterpart [8]. The interlamellar space has been characterized as having a complex fibrous network between lamellae [9]. Additionally, distinct fibrous bridging ele-

ments have been shown to span across multiple lamellae, complicating investigations that seek to describe the microstructure of the AF [10]. The mechanical contribution of the interlamellar region and the bridging elements has not been comprehensively characterized. The alternating fiber orientation in adjacent lamellae may lead to high interlamellar shear during physiological loading of the disk [11], and this shear may contribute to the overall tensile stiffness of the AF [12]. The interlamellar cellular deformations resulting from this shear may play an important role as a mechanically triggered repair mechanism for the AF. Characterizing the link between local tissue deformations and biological repair would provide important information for the design of tissue engineering technologies, as well as improve our understanding of degenerative disk disease.

Finite element (FE) modeling is a useful tool for predicting physiological loading environments [13–17]. Predictions of relative deformations and local stresses within the lamellar and interlamellar regions resulting from physiologically relevant loading conditions could be used to guide experiments that link biological response to local deformation (mechanobiology). However, FE model predictions are dependent upon two key inputs: geometry and material definitions. The material properties used in a model are typically obtained via physical mechanical testing of tissue specimens. AF lamellar mechanics have been well characterized previously via biaxial tension, compression, and shear testing [18–20,23]. Computational models derived from these experiments have treated the AF as a homogeneous tissue with two families of fibers rather than describing the mechanics of the lamellar and interlamellar spaces individually. It has been shown that this description is sufficient for modeling global AF tissue behavior

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under a variety of loading conditions [18–20], but it does not provide specific information with respect to interlamellar mechanics. There is also limited experimental data describing the relatively high displacements of adjacent lamellae under shear loading [9,24]. Therefore, the purpose of this study was to describe the stress–strain response of the lamellar and interlamellar components via a simple shear loading experiment of the ovine AF. Data were collected for multiple regions of the disk and fit to a constitutive continuum mechanics formulation for implementation into a finite element model of the lamellar-interlamellar construct.

Methods

Interlamellar Shear Experiments. Ovine lumbar intervertebral disks were dissected from four spines obtained from animals euthanized for other purposes. Rectangular specimens were isolated from three regions of the AF: inner anterior, outer anterior, and lateral (Fig. 1). Eighteen specimens were harvested from each location. Specimens were cut into approximately 9 mm long (circumferential) by 4 mm wide (radial) by 3 mm thick (axial) tissue blocks. Prior to testing, the cross-sectional area of the specimens was measured via digital image analysis for post hoc stress calculations [18]. Specimens were attached to two acrylic platens with cyanoacrylate on their inner and outer radial surfaces for placement into the testing apparatus (Fig. 1). The use of a gel-like cyanoacrylate ensured that the adhesive did not penetrate into the tissue from the periphery such that the central region of interest was not affected by this fixation method. Residual stresses induced by fixing the slightly curved specimens onto a flat surface were assumed to be minimal. India ink markers were applied to the surface of each specimen in order to track relative tissue stretch during the experiment. A minimum of six markers within a

single lamella and three markers on the adjacent lamella were tracked. Adjacent AF lamellae have alternating fiber orientations, and therefore, the layers of the laterally illuminated specimen were optically distinct, ensuring accurate placement of the fiducial markers. Markers were placed in the central lamellae of the specimen to eliminate boundary/fixation (St. Venant's) effects.

Specimen hydration was ensured throughout the preparation and testing procedures with periodic saline spray. One specimen platen was rigidly secured to a 44 N (10 lb) load cell (Model 31, Honeywell Sensotec, Columbus, OH), and the other platen was displaced with a linear actuator (ATS50, Aerotech, Pittsburgh, PA) such that the specimen was loaded in simple shear. Based upon a series of pilot experiments that demonstrated tissue behavior convergence, specimens were preconditioned to 0.75 mm displacement for five cycles at a rate of 0.02 shear strain per second. Specimens were then returned to a baseline force of 0 N and ramped to 1 mm displacement. In order to minimize temporal (i.e., viscoelastic) effects, the experimental protocol utilized quasi-static loading with a strain rate of 0.01 s^{-1} [18,24]. Specimen surface images were captured with a CCD camera (Flea3, Point Grey Research, Richmond, BC, Canada) in conjunction with a stereo microscope (Steromaster, Fisher Scientific, Waltham, MA), yielding a spatial resolution of $3 \text{ }\mu\text{m}/\text{pixel}$. Images were analyzed using a custom-written code in MATLAB (Mathworks, Natick, MA) to track the fiducial marker centroids. The two-dimensional deformation gradient tensor F was calculated from each triad of markers and averaged for triads within a single lamella and across the interlamellar layer (Fig. 2). Deformations were calculated using two-dimensional Green–Lagrangian strain (E)

$$E = \frac{1}{2}(C - I)$$

Fig. 1 (a) Rectangular AF specimen blocks were isolated from three locations of the disk. (b) Schematic demonstrating the simple shear experiment with fiducial marker placements (black dots). (c) Digital image of the overall experimental apparatus that utilized a microscope to track marker displacements. (d) Close-up view of the shear experimental apparatus.

Fig. 2 Specimen image demonstrating the AF's distinct layers. Lamellar and L + IL shear were defined by marker triads within a lamellae and across the interlamellar space, respectively.

where I is the identity matrix and C is the right Cauchy Green strain tensor

$$C = F^T F$$

Because the markers used to define shear across the interlamellar layer were located within the adjacent lamellae, the data described the combined shear behavior of the lamellar and interlamellar layers. Therefore, the two experimental measurements have been defined as lamellar and lamellar plus interlamellar (L + IL).

Curve Fitting to a Strain Energy Density Function. For incorporation into the finite element model, the lamellar and L + IL material properties were modeled using a hyperelastic strain energy density function defined in terms of the scalar strain invariants. An isotropic, two-term Yeoh formulation was chosen to model the L + IL material. The L + IL strain energy density function was defined as

$$W_{L+IL} = C_1(I_1 - 3) + C_2(I_1 - 3)^2$$

where the coefficients C_1 and C_2 represent twice the initial shear modulus and nonlinearity, respectively, and I_1 is the first strain invariant. The coefficients were determined by fitting the function to the shear stress–stretch data by using a custom-written MATLAB code. A MATLAB function (fmincon) was utilized to minimize the sum of squared residuals. The lamellar material was defined by an anisotropic, three-term strain energy density function (W_{Lamellar}) that superimposed the strain energy contributions of the matrix (W_{matrix}), fiber (W_{fiber}), and shear terms (W_{shear})

$$W_{\text{Lamellar}} = W_{\text{matrix}} + W_{\text{shear}} + W_{\text{fiber}}$$

The matrix term (W_{matrix}) was modeled using an isotropic, two-term Yeoh model to describe the ground substance

$$W_{\text{matrix}} = C_1(I_1 - 3) + C_2(I_1 - 3)^2$$

The coefficients used for the matrix term were taken from previously published work [18]. The shear term was used to describe the shear interactions between aligned fibers within a lamella [19]. The shear contribution was modeled using the following formulation:

$$W_{\text{shear}} = \frac{A_1}{A_2} e^{(A_2(I_5 - I_4^2))}$$

where the coefficients A_1 and A_2 represent the linear and nonlinear shear behavior, respectively. I_4 and I_5 represent the fourth and

Fig. 3 Finite element model of AF block, with simple shear loading conditions matching experiments. Element dimension was 180 μm in the axial (out of plane) direction.

fifth anisotropic strain invariants, respectively [25]. The coefficients were determined by fitting the combined matrix and shear terms to the stress–deformation relationship from the shear experiments. The fiber term described the anisotropic, hyperelastic behavior of the collagen fibers within a lamella and is commonly used to model the AF [18–20]

$$W_{\text{fiber}} = \frac{B_1}{B_2} (e^{(B_2(I_4 - 1)^2)} - 1)$$

where the coefficients B_1 and B_2 represent the linear and nonlinear fiber behavior, respectively. The fiber coefficients were determined by fitting previously reported biaxial tension data [18] in the circumferential–axial plane of the AF to the full three-term strain energy density function. The theoretical Cauchy stresses (σ) used for fitting the experimental data were calculated by numerical differentiation of the strain energy density functions

$$\sigma = 2J^{-1} F^T \frac{dW}{dC} F$$

The determinant of the Jacobian was set to unity due to the assumption of incompressibility Wu [21].

Finite Element Modeling. A simple finite element model of a block of AF tissue was created to match the average geometry of the specimens used in the shear tests (9.0 mm × 2.5 mm × 3.3 mm) and contained eight lamellae through its thickness. The model was created and analyzed using ABAQUS (ver. 6.11, Simulia, Providence, RI), and the model utilized hexahedral elements with a hybrid formulation to control hourglassing and allow for the assumption of incompressibility. The lamellar and L + IL materials were represented distinctly, utilizing a continuum description for each layer (Fig. 3). The thicknesses of the L + IL and lamellar layers were 0.19 mm and 0.23 mm, respectively. The L + IL thickness was based on the average measured perpendicular distance between markers used to experimentally measure the L + IL shear. The overall layer thickness of 0.42 mm was based on average histological measurements of lamellar thickness of nine ovine lumbar disks. The strain energy density functions described above were incorporated into the model via a custom-written user subroutine in ABAQUS (uanisohyper_inv).

The model was analyzed in simple shear to match the loading and boundary conditions of the physical experiments described above. The bottom surface of the model was kinematically constrained, and a lateral (shear) displacement boundary condition was applied to the top surface. The resultant force was computed, and the global stress was calculated by dividing this force magnitude by the cross-sectional area. The shear term of the deformation gradient was calculated as the displacement of the top surface divided by the block height, and the Green–Lagrangian strain (E) was subsequently calculated using Eq. (1). The average stress–strain relationship was compared between the model predictions and experiment for the AF block.

Statistical Analysis. The initial and final shear moduli were calculated for each specimen and compared across groups. The

Fig. 4 Characteristic L + IL and lamellar experimental stress-strain curves from a single specimen (outer anterior) demonstrating the constitutive model's fit to the experimental data.

initial and final moduli were defined as the slopes from $E=0-0.01$ and over the final range of 0.01 strain, respectively. Experimental lamellar and L + IL data and regional groups were compared using a two-sided Welch's t-test, with significance set at $p < 0.05$ (R 1.15.1, The R Foundation for Statistical Computing). Due to the skewed, non-Gaussian distribution of the constitutive equation coefficients, the median coefficients determined from fitting the physical experiments were incorporated into the finite element material definitions.

Results

Shear Experiments. The AF in simple shear exhibited a non-linear stress-strain relationship for both the L + IL and lamellar layers at each location tested, which could be accurately described by the chosen strain energy density functions (Fig. 4). It was typical for the L + IL layer to exhibit greater strains than the lamellar layer of a given specimen. The constitutive models were able to describe the data well, with a mean coefficient of determination of 0.99 and a range of $0.91 < R^2 < 0.99$. The constitutive equations containing the median coefficients (Table 1) were incorporated into the finite element model.

Adjacent lamellae were observed to slide relative to each other in most specimens, resulting in greater L + IL shear strain than lamellar shear strain (Fig. 4). Comparison between L + IL and lamellar compartments (Fig. 5) revealed that the mean shear moduli at the initial and final regions of the curve were significantly greater for the lamellae than the L + IL at the outer anterior and lateral regions. However, the difference was not significant at the inner anterior region. The ratios of L + IL/lamellar initial shear modulus for the outer anterior, inner anterior, and lateral locations were 0.51, 0.90, and 0.52, respectively. For the final shear modulus the same ratios for the outer anterior, inner anterior, and lateral locations were 0.56, 0.79, and 0.57, respectively. Comparison of the data means between groups (Fig. 5) showed that the shear moduli were significantly different between locations of the disk, with the outer anterior having the highest stiffness, followed by the lateral, and inner anterior regions. This was the case for both the initial and final shear moduli. For each group, the final shear modulus was greater than the initial shear modulus, demonstrating the classic hyperelastic behavior of the tissue.

Finite Element Modeling. Comparison between analytical solutions and the local stress-deformation relationship of individual elements in the model gave less than a 3% difference for each group, indicating that the user subroutine material definitions accurately described the material behavior in the finite element analysis. The finite element analysis of the global AF shear replicated the nonlinear stress-strain relationship that was seen

Table 1 Median coefficients from strain energy density functions

		Region		
		Outer anterior	Inner anterior	Lateral
L + IL	C1 (kPa)	50.7	13.7	23.5
	C2 (kPa)	212	47.5	53.2
Lamellar	C1 (kPa)	15.4	15.4	15.4
	C2 (kPa)	74.3	74.3	74.3
	A1 (kPa)	330	24.3	79.9
	A2	32.4	17.7	16.2
	B1 (kPa)	2230	2230	2230
	B2	49.0	49.0	49.0

A. Initial Shear Modulus

B. Final Shear Modulus

Fig. 5 Shear moduli at the initial (a) and final (b) regions of the stress-strain curve for the three AF locations. Data represent mean with standard deviation error bars. Similar letters indicate statistical relationships: (A) $p = 0.01$; (B) $p = 0.63$; (C) $p = 0.034$; (D) $p < 0.01$; (E) $p = 0.018$; (F) $p < 0.01$; (G) $p < 0.01$; (H) $p = 0.015$; (I) $p = 0.15$; (J) $p < 0.01$; (K) $p < 0.01$; (L) $p = 0.041$; (M) $p < 0.01$; and (N) $p < 0.01$.

experimentally (Fig. 6). For each of the three locations, the global stress-strain curves of the model fell within one standard deviation of the experimental mean (Fig. 6), with the inner anterior model matching the experimental data. However, the outer anterior and lateral models underpredicted the shear stress at higher shear strains.

Because the model was loaded in shear, the fiber tension was not developed within the center of the specimen. There was some fiber tension in the corners due to boundary effects; however, the fibers' contribution to the total strain energy was negligible (less than 1%). In the lamellar compartment, the contributions of the matrix and shear components to the strain energy were 77% and 23%, respectively, for the outer anterior; 70% and 30%,

Fig. 6 Comparison of global stress-strain relationship between the model prediction and experimental data. (a) Outer anterior, (b) inner anterior, and (c) lateral.

respectively, for the inner anterior; and 70% and 30%, respectively, for the lateral location.

Discussion

This study has characterized the in vitro mechanical behavior of the lamellar and interlamellar regions of the AF in

circumferential-radial shear. Data from experimental shear tests were incorporated into strain energy density functions, and these functions were implemented into a computational model of the lamellar-interlamellar construct. Regional mechanical differences were compared between lamellar and L + IL layers, as well as across various locations of the intervertebral disk. Overall, greater shear deformation (i.e., compliance) was seen in the L + IL layer than within lamellae. It was observed that adjacent lamellae appeared to slide relative to each other, which is supported by the data showing higher lamellar shear moduli. This result is not surprising, considering that the interlamellar compartment can undergo extremely high deformations in vitro [24], and the microstructural organization of the AF promotes high interlamellar shear [9]. However, AF shear has previously been reported to include no interlamellar sliding [26]. The discrepancy between results may be due to the difference in specimen orientation with respect to the lamellar fiber directions. Differences in animal models (ovine lumbar spines versus bovine tails) may also account for these conflicting results.

Differences in shear moduli were seen between the various locations of the disk. Of particular interest, the outer anterior AF had much higher stiffness than the inner anterior. This is in agreement with previous studies that have shown regional variations in AF mechanical behavior [27,28]. The difference in mechanical properties between inner and outer AF is likely related to their relative biochemical compositions; the outer AF has a higher collagen content, and the inner AF has a higher glycosaminoglycan (GAG) content [29–31] (similar to the GAG-rich nucleus pulposus). Microstructural differences between the inner and outer AF have also been reported, with changing collagen fiber angles having been observed as one traverses the AF radially [31,32]. Additionally, the difference between lamellar and L + IL shear behavior was significant in the outer anterior AF, but these layers behaved similarly at the inner anterior region of the AF (Fig. 5). This result seems to suggest that the interlamellar region may have a more distinct mechanical role in the outer AF.

The mean data demonstrated higher deformations within the L + IL layer than within lamellae, but this was not the case for every specimen that was evaluated in this study. Interspecimen data variability is likely related to the complex microarchitecture of the AF. The wide range of fibrous network connections and bridging elements [9,10] produce a highly interconnected structure and heterogeneous mechanical environment. Therefore, the results presented herein describe the average mechanical contribution of these interlamellar connections in shear, which is useful for continuum modeling the AF but does not provide a fully detailed description of interlamellar mechanical behavior.

The description of L + IL shear presented here should not be misinterpreted as a pure definition of the material between adjacent lamellae. Rather, the interlamellar shear behavior as described in the current study is limited by the spacing of the fiducial markers, which were measured to have a mean perpendicular distance of 0.19 mm across the interlamellar layer. The markers were located in adjacent lamellae, not within the actual interlamellar compartment. Therefore, the data describe the shear across the interlamellar layer and include information about the shear properties of each lamella in addition to the interlamellar compartment. With higher resolution deformation data that could isolate the interlamellar compartment from the lamellae, the coefficients for the constitutive equation may differ from those reported here. Nevertheless, the form of the constitutive equation was still shown to describe the data well and provides quantitative information with respect to the relative mechanical behavior of the interlamellar and lamellar components of the AF.

The L + IL constitutive model utilized an isotropic form. In order to capture any anisotropy that may be present, further experiments would need to be performed that include additional loading cases. Distinguishing the compressive or tensile properties of the interlamellar layer from the lamellae could prove challenging due to the small, nonuniform thickness of the layer. However,

if one is primarily interested in studying the interlamellar layer in shear, the constitutive model presented is sufficient for describing the interlamellar material for use in finite element analysis. It should be noted that the matrix and fiber terms of the strain energy equation were taken from previous work on human tissue that was not location specific [18]. Therefore, the coefficients for these terms were the same at each location and do not necessarily reflect the properties of the specimens tested in the current study. However, the shear term was fitted together with the already determined matrix term to the experimental data, and thus, the constitutive model still accurately describes the shear behavior.

The finite element model of the AF block predicted the experimental global shear within one standard deviation of the mean. This correspondence supports the approach derived herein of modeling the AF with distinct interlamellar and lamellar layers with the selected material definitions for accurately describing the shear mechanics of the tissue. The outer anterior location, which exhibited the greatest difference between lamellar and L + IL stiffness, had the greatest difference between model and experimental mean. The outer anterior model also had the smallest contribution of the shear term to the strain energy, relative to the matrix term, which was unexpected because the shear term coefficients are greatest for the outer anterior. We observed that the outer anterior model predicted greater out-of-plane shear deformations within lamellae, which effectively reduced the relative contribution of the shear term as the elements deformed to find their lowest energy state for the given loading condition and magnitude.

Sheep models have proven to be useful tools in spine research [33–35]. An ovine finite element model of an intervertebral disk can be used to complement in vivo animal studies involving spine mechanics. Ovine lumbar spines are anatomically [36] and biomechanically [37] similar to human spines. Specifically, ovine and human lumbar intervertebral disks have similar water content, collagen content, and fiber angles [31]. Due to the basic structural similarities, it is likely that human AF specimens would exhibit similar behavior in shear to the results from the current study. Given the results reported herein, future studies on human AF shear are warranted.

The data reported herein can be used for creating an intervertebral disk finite element model that describes interlamellar and lamellar regions discretely. Such a model could be useful for predicting the relative local deformations in the interlamellar and lamellar regions for physiologically relevant loading cases. Predictions of local interlamellar deformations could be used to inform experiments that seek to investigate interlamellar mechanobiology. Previous experimental approaches have studied cellular mechanics under in situ loading conditions [38], or provided a controlled environment to apply prescribed deformations to cell culture systems [39]. Combining these approaches with finite element predictions, with a focus of interlamellar cell mechanics, could give a full description of the biological response of interlamellar cells to loading of the intervertebral disk. Ultimately, this information could aid in the understanding of degenerative disk disease and enhance the design of disk regeneration therapies.

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