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Mechanisms for mechanical damage in the intervertebral disc annulus fibrosus

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Abstract

Intervertebral disc degeneration results in disorganization of the laminate structure of the annulus that may arise from mechanical microfailure. Failure mechanisms in the annulus were investigated using composite lamination theory and other analyses to calculate stresses in annulus layers, interlaminar shear stress, and the region of stress concentration around a fiber break. Scanning electron microscopy (SEM) was used to evaluate failure patterns in the annulus and evaluate novel structural features of the disc tissue. Stress concentrations in the annulus due to an isolated fiber break were localized to approximately 5 μm away from the break, and only considered a likely cause of annulus fibrosus failure (i.e., radial tears in the annulus) under extreme loading conditions or when collagen damage occurs over a relatively large region. Interlaminar shear stresses were calculated to be relatively large, to increase with layer thickness (as reported with degeneration), and were considered to be associated with propagation of circumferential tears in the annulus. SEM analysis of intervertebral disc annulus fibrosus tissue demonstrated a clear laminate structure, delamination, matrix cracking, and fiber failure. Novel structural features noted with SEM also included the presence of small tubules that appear to run along the length of collagen fibers in the annulus and a distinct collagenous structure representative of a pericellular matrix in the nucleus region.

Keywords

Intervertebral disc; Annulus fibrosus; Collagen fiber damage; Interlaminar shear stress; Delamination

1. Introduction

Intervertebral disc degeneration is associated with mechanical damage, loss of nutritional pathways and biological degradation. We believe that mechanical damage propagation in the intervertebral disc is a primary factor distinguishing disc degeneration from age-related changes in the disc. Understanding mechanical failure mechanisms in the annulus due to

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loading will allow researchers to predict damage propagation and to isolate mechanical damage from biological degradation in the degeneration process.

Mechanical overloading from hyperflexion, torsion, and fatigue loading was considered a potential cause of disc failure (Farfan et al., 1970; Adams and Hutton, 1982, 1983; Yu et al., 2003). Annulus fibrosus tears include radial tears (perpendicular to the endplates and cut through the annulus layers), circumferential tears (ruptures between annulus layers along the circumference of the disc), and rim lesions (radial tears at the periphery of the annulus adjacent to endplates) (Vernon-Roberts, 1987; Thompson et al., 1990). Failure initiation and propagation of all three types of tears was simulated with compressive and bending loads (Natarajan et al., 1994). Interlaminar shear stresses and separation of layers in the annulus were also predicted to be a cause of failure propagation in the disc in the important study by Goel et al., using a three-dimensional finite element model of a spinal motion segment incorporating a composite annulus fibrosus (Goel et al., 1995).

The solid matrix of the intervertebral disc is organized into a gelatinous nucleus pulposus and highly organized angle-ply laminate structure of the annulus fibrosus. The laminate structure and fiber angle of the annulus fibrosus has been the subject of careful study using light microscopy, X-ray diffraction, electron microscopy, and stereophotogrammetry techniques (Inoue and Takeda, 1975; Hickey and Hukins, 1980, 1981; Inoue, 1981; Stokes and Greenapple, 1985; Cassidy et al., 1989; Marchand and Ahmed, 1990; Bernick et al., 1991; Tsuji et al., 1993). The annulus has a regular angle-ply laminate structure with the number of layers, thickness of layers, and number of irregularities in the laminate structure varying with region in the disc and with aging (Cassidy et al., 1989; Marchand and Ahmed, 1990; Tsuji et al., 1993). In addition, the interlamellar fiber angle ranged between 45° and 25° (from the transverse plane) from inner to outer annulus region (Cassidy et al., 1989).

Material properties of the intervertebral disc annulus fibrosus in tension, compression, shear, and swelling behaviors were influenced by disc region, specimen orientation, and level of aging and degeneration, and fatigue loading (Galante, 1967; Wu and Yao, 1976; Panagiotacopoulos et al., 1979; Best et al., 1994; Skaggs et al., 1994; Acaroglu et al., 1995; Ebara et al., 1996; Fujita et al., 1997, 2000; Gu et al., 1999; Iatridis et al., 1998, 1999, 2003; Klisch and Lotz, 1999; Elliott and Setton, 2001). Theories relating the microstructure of the annulus with material properties involved application of hyperelastic analysis with linear or exponential strain energy functions describing material orthotropy (Wu and Yao, 1976; Klisch and Lotz, 1999; Elliott and Setton, 2000; Eberlein et al., 2001). Finite element models with fiber elements and laminated structures also incorporated fiber reinforcement with different techniques, e.g., (Kulak et al., 1976; Shirazi-Adl, 1989a, b; Natarajan et al., 1994; Goel et al., 1995). Material properties and laminate structural characteristics are available for the intervertebral disc, although research and technology advances make their determination an ongoing process. Composite models provide relevant tools to evaluate the influence of fiber orientation angle and laminate structure on mechanical behaviors and failure mechanisms in the annulus fibrosus.

We hypothesize: (1) multiple failure patterns in the annulus fibrosus occur simultaneously due to the composite laminate structure, and (2) alterations in the laminate structure

commonly seen with degeneration (i.e., increase in layer thickness, focal disruption or existing cracks) will increase interlaminar shear stresses and may provide a mechanism for damage propagation in the annulus. To test the hypotheses, we performed a three-part study. The first part of this study was a stress analysis on the intervertebral disc annulus fibrosus using composite lamination theory and experimental data in the literature. The second goal of this study was to evaluate the roles of fiber failure and delamination (i.e., separation of annulus layers) in contributing to composite failure by calculating the ineffective length of a broken fiber and interlaminar shear stress. The third goal was to evaluate failure patterns and important microstructural features in the annulus fibrosus using scanning electron microscopy (SEM).

2. Methods

2.1. Lamination theory analysis of the angle-ply annulus fibrosus

Substantial work in composite material lamination theory provided a framework for analysis of the angle-ply laminated structure of the annulus fibrosus. In lamination theory, the assumption of plane stress and knowledge of the predominant fiber angle allow development of constitutive rules that relate the normal stresses and bending moments to the mid-plane strains and curvatures of a laminated plate, as given in many texts on composite materials, e.g., (Swanson, 1997; Jones, 1999). The terms relating the stresses to the strains are given by three stiffness matrices (in-plane stiffness matrix, bending stiffness matrix, and coupling stiffness matrix relating in-plane strains and curvatures to in-plane forces and bending moments). These stiffness relationships are functions of the laminate geometry, elastic material constants of the fiber and matrix, fiber volume fraction, and fiber orientation angle. The six material constants required for the linearly elastic plane stress analysis are: fiber modulus (E_f), matrix modulus (E_m), in-plane Poisson's ratio (ν_{12}), in-plane shear modulus (G_{12}), fiber volume fraction (V_f), fiber angle (θ) and number and thickness of layers. *Longitudinal* and *transverse* properties, stresses and strains of a lamina are oriented in the one-direction (parallel to the fibers) and two-direction (perpendicular to the fibers), respectively (Fig. 1). *Fiber* and *matrix* (subscripts f and m) properties, stresses, and strains may then be calculated from these longitudinal and transverse properties based on certain microstructural assumptions and lamination theory. Anatomic directions *circumferential*, *axial*, and *radial* correspond to the x , y , and z directions, respectively (Fig. 1). As we only analyze rectangular test specimens in this manuscript, the Cartesian coordinate system is appropriate.

To obtain the full set of material constants and validate the model, structural and material property data available in the literature were used to estimate E_f , E_m , ν_{12} ; and V_f , and experimental data for uniaxial tension (Table 1). Lamination theory was then used to calculate values for fiber angle θ and in-plane shear modulus G_{12} , which were then compared with values in the literature. Fiber and matrix volume fractions were determined to be $V_f = 0.15$ and $V_m = 0.85$ based on measurement of collagen content per dry weight (60% dry weight) and water content of 75% (Skaggs et al., 1994) and is similar to that previously calculated (Marchand and Ahmed, 1989). The elastic modulus of a single annulus layer in the longitudinal direction, E_l , was 136 MPa (Skaggs et al., 1994) and the elastic

modulus of the matrix, E_m , was estimated based on compressive material properties of the annulus to be 0.5 MPa (Iatridis et al., 1998) and is similar to radial tensile properties (Fujita et al., 1997). The elastic modulus of collagen fibers, $E_f = 904$ MPa was calculated using the rule of the mixtures (i.e., $E_f = (E_1 - E_m V_m) / V_f$). A parametric study demonstrated insensitivity of the in-plane stiffness matrix to ν_{12} which was taken to be 0.3 since it is unavailable in the literature. The Halpin–Tsai relationship was used to obtain modulus transverse to the fibers (E_2) from fiber and matrix properties (Jones, 1999). Parameters θ and G_{12} are deeply embedded in the stiffness equations, and therefore a nonlinear regression technique (fminsearch, MATLAB, Natick, MA) was used to solve for these constants based on experimental data found in the literature.

Evaluation of the tensile stress and strain in the circumferential and axial directions of the laminated structure were taken from the anisotropic tensile material properties previously reported (Acaroglu et al., 1995; Elliott and Setton, 2001). The laminate was assumed a symmetric angle-ply laminate with eight layers (see *Interlaminar shear stresses and delamination*). Using material constants for the tangent modulus and Poisson's ratio oriented in an anatomical coordinate system available in the literature, the experimental data was compared to lamination theory to solve for θ and G_{12} under four different loading cases: (1) circumferential tension (Acaroglu et al., 1995), and (2) circumferential tension (Elliott and Setton, 2001), (3) axial tension (Elliott and Setton, 2001), and (4) circumferential and axial tension (i.e., applied separately) (Elliott and Setton, 2001).

Once the full set of six material constants were obtained, an analysis was performed to estimate the stresses in the 1–2 and x – y coordinate systems within each layer of the laminated plate when subjected to a tensile strain of 0.10 in the x -direction (circumferential direction of the annulus), according to standard procedures in lamination theory.

2.2. Interlaminar shear stresses and delamination

The presence of stress-free boundaries on a laminated material introduces interlaminar stresses in the vicinity of the free edges (Swanson, 1997; Herakovich, 1998). Stress-free boundaries are an essential component of a uniaxial tension test; however, in the intact intervertebral disc, free edges in the annulus occur near focal disruptions or existing cracks that might exist from degenerative changes or mechanical damage. For a free-body diagram of a general angle-ply laminate loaded in the x -direction (Fig. 1b), the equation of equilibrium in the x -direction is

$$\frac{\partial \sigma_x}{\partial x} + \frac{\partial \tau_{xy}}{\partial y} + \frac{\partial \tau_{xz}}{\partial z} = 0, \quad (1)$$

where σ_x is the axial stress, τ_{xy} is the in-plane shear stress, and τ_{xz} is the interlaminar shear stress (Fig. 1a). Assuming the stresses are uniform along the x -direction, Eq. (1) may be simplified and integrated with respect to z :

$$\tau_{xz} = - \int_{-h/2}^z \frac{\partial \tau_{xy}}{\partial y} dz, \quad (2)$$

where h is the full laminate thickness. Equilibrium, therefore requires that unbalanced in-plane stresses (τ_{xy}) must be balanced by interlaminar stresses (τ_{xz}). For a uniaxial tension specimen, the stress τ_{xy} which is nonzero at $\theta = 0$ must vanish on the free edge shown (planes $\theta = \pm h$; due to the stress-free boundary conditions on the free surfaces shown in Fig. 1b). The stress gradient of in-plane shear stress τ_{xz} with θ is a maximum at the outer edge of the boundary where the interlaminar shear stress τ_{xz} is largest. The values for interlaminar stresses τ_{xz} were approximated based on results from lamination theory, as described (Rose and Herakovich, 1992; Herakovich, 1998). To assess the effect of layer thickness and number of layers on interlaminar shear stress, τ_{xz} ; two symmetric angle-ply laminates were used: an eight-layer $[(-\theta' + \theta_2)_s]$ layup and a four-layer $[-\theta' + \theta]_s$ layup with twice the thickness per layer. The layer thickness was taken to be 0.2 mm (with half-layers at the top, bottom, and middle surfaces to maintain z -axis symmetry for the layup) for the eight-layer layup and 0.3 mm for the four-layer layup which is similar to thickness values reported for outer annulus from young and old humans, respectively (Marchand and Ahmed, 1990). The fiber angle, θ , was taken from the curve fit in the laminate analysis for uniaxial tension.

2.3. Stress in a collagen fiber near a fiber break

A stress concentration exists next to a collagen fiber break because the broken fiber does not carry load and adjacent fibers must compensate by carrying more load. However, due to shear stresses carried in the matrix along the length of the fiber, the stress in the fiber will resume to nominal levels a finite distance from the break. An equilibrium structural analysis modified from Swanson (1997) was performed to estimate the axial distance along a collagen fiber required for the load to be restored to the fiber after a break in the fiber. We first assume there is a group of collagen fibers with a fiber break (Fig. 2a). The fiber is assumed to be idealized as a simple axial rod under tension with shear transmitted into the fiber from the surrounding matrix (Fig. 2b). Equilibrium for the segment of fiber requires:

$$\left(\sigma_f \frac{\partial \sigma_f}{\partial x_1} dx_1 \right) A_f - \sigma_f A_f - \tau_m C_f dx_1 = 0, \quad (3)$$

where σ_f is the axial stress in the fiber, $A_f = \pi r_f^2$ is the cross sectional area of the fiber, $C_f = 2\pi r_f$ is the circumference of the fiber, r_f is the radius of the fiber, and in this analysis, the fibers are oriented with the 1-axis, which is denoted as dx_1 to represent a differential length oriented in the 1-axis direction. Combining terms in Eq. (3) and dividing by the common term, dx , leads to

$$\left(\frac{\partial \sigma_f}{\partial x_1} \right) A_f - \tau_m C_f = 0. \quad (4)$$

Fibers and matrix are assumed linearly elastic ($\sigma_f = E_f \epsilon_f$ and $\sigma_m = G_m \gamma_m$) where E_f and G_m , are the Young's modulus of the fiber and shear modulus of the matrix, respectively. Assuming small strains, the shear strain $\gamma_m = (u_f - u)/h$ and axial strain $\epsilon_0 = u/x_1$ in the matrix are defined where u is the displacement of the surrounding matrix far away from the break, ϵ_0 is the nominal strain field far away from a break, u_f is the displacement of the broken fiber far away from the break, and h is the distance between fibers. The strain in the

fiber is given as $\varepsilon_f = du_f/dx_1$. Plugging stress–strain and strain–displacement relationships into Eq. (4) gives

$$\frac{\partial \varepsilon_f}{\partial x_1} = \frac{2G_m}{E_f r_f h} (u_f - u), \quad (5)$$

which can be simplified to the following ordinary differential equation in terms of u_f :

$$\frac{\partial^2 u_f}{\partial x_1^2} - \beta^2 u_f = -\beta^2 \varepsilon_0 x_1, \quad (6)$$

where $\beta^2 = 4G_m/(E_f D_f h)$, and D_f is the fiber diameter. Values for the shear modulus of the annulus are 0.1–0.5 MPa (Iatridis et al., 1999), values for Young's modulus of the fibers were 904 MPa based on rule of mixtures and (Skaggs et al., 1994), and the collagen fiber diameter ranged from 50 to 150 nm with fiber separation (h) of approximately $r_f/2$ based on the TEM images (Iatridis et al., 2003). The value for β is approximately $0.28/D_f$ and gets smaller as the difference between the shear and tensile moduli increases. Boundary conditions indicated that stress in the fiber is zero at the fiber break ($x = 0$) and the displacement in the fiber u_f remains finite for large x_1 . Solving, we obtain the following relationship for stress in the fiber:

$$\sigma_f = E_f \varepsilon_0 (1 - e^{-\beta x_1}). \quad (7)$$

From this relationship, we obtained the distance x_1 that it takes before the fiber is carrying most of the load (i.e., 95% of value of unbroken fiber), thereby defining the *ineffective length* of the broken fiber.

2.4. Scanning electron microscopy

SEM of four intervertebral discs from tails from two mature (B1 y.o.) Wistar rats was used to evaluate failure patterns and other microscopic features in the nucleus and annulus regions. Within 1 h of death, skin was removed from the tails of mature (~1 y.o.) control rats, vertebra–disc–vertebra sections were isolated with a razor cut through the middle of the adjacent vertebrae, tail motion segments were immediately frozen by plunging in liquid propane for 2 min before being transferred to liquid nitrogen for further processing. Specimens were then fractured in the sagittal plane under liquid nitrogen followed by a freeze-substitution process in acetone, for 7 days at 193 K, critical point drying, and sputter coated to provide a 10 nm Pt/Pd surface coating as previously described (ap Gwynn et al., 2000). High-resolution SEM were taken on a Hitachi s-4700 FESEM, using the low accelerating voltage, high current, and backscattered electron imaging method described previously (Richards et al., 1999).

3. Results

3.1. Lamination theory analysis of the angle-ply annulus fibrosus

Values for fiber angle and shear modulus G_{12} obtained from the regression analysis were insensitive to initial guess suggesting we converged on a unique solution. However, simulated testing conditions did impact results with fiber angles of 38° , 34° , 39° , and 39° and G_{12} of 2.91, 2.19, 0.277, and 0.291 MPa for loading cases 1–4, respectively. The values $\theta = 39^\circ$ and $G_{12} = 0.3$ MPa from Case 4 were considered most accurate since they were closest to previously reported values and obtained from tensile data with two orientations of testing, and therefore used for further stress analyses.

Under 10% strain applied in the x -direction (circumferential hoop strain), x -direction stresses had the largest magnitude but in-plane shear stress and stress in y -direction were both quite large (Fig. 3a). The oscillation of in-plane shear from positive to negative values with z -direction was representative of the discontinuous values for shear modulus from layer to layer. When viewed in the material coordinate system (i.e., 1–2 coordinates, Fig. 3b), it was highly evident that fibers dominated load carriage in tension. The longitudinal stress was approximately 8 MPa while transverse and in-plane shear (1–2) contributions had magnitudes less than 0.1 MPa due to the very high value for E_1 relative to E_2 (even though strains in the longitudinal and transverse direction were much closer in value for this strain controlled simulation with values of 6% and 4% in the longitudinal and transverse directions, respectively). The maximum stress in the fibers and matrix (based on 6% longitudinal strain and fiber and matrix moduli) were 54 and 0.03 MPa, respectively.

3.2. Interlaminar shear stresses near a free edge

Under tensile loading of the annulus in the circumferential direction, distributions of interlaminar shear stress (Fig. 4) demonstrated that τ_{xz} is linear through each layer and exhibits identical maximum magnitudes at each $\pm\theta^\circ$ interface. As the width of each layer doubles (and associated number of layers is halved), the magnitude of the interlaminar shear stress is greatly increased.

3.3. Stress in a collagen fiber near a fiber break

The model of bridging around a fiber break demonstrated that for a value of βx_1 of three (i.e., $x_1 = 32D_f$), the fiber stress was 95% of its original value and the fiber behaved as a nearly unbroken fiber. Therefore, the *ineffective* length of the broken fiber was approximately 5 μm away from the fiber break, where the broken fiber was nearly as effective as an unbroken fiber in transmitting tensile load.

3.4. Scanning electron microscopy

SEM images produced from freeze fracturing and freeze substitution clearly showed distinct collagenous structural patterns in the nucleus pulposus (Fig. 5) and the annulus fibrosus (Fig. 6). In the nucleus, a fine collagenous network with random fiber orientation was observed. Also of interest was the observation of distinct spherical voids of densely packed and fine collagen, which are presumably the pericellular matrix where cells had previously resided. In the annulus, a laminate structure was clearly present with 15–20 μm -thick layers

of alternating interlamellar angles. Each layer was comprised of collagen fibers of relatively large diameter that contained many fine fibrils. At the fracture site, evidence of separation of adjacent layers (delamination) as well as separation of the fibers within each layer (matrix cracking) was also present. An additional finding was that the collagen in the annulus fibrosus of rat intervertebral disc tissue formed distinct tubules running parallel to the fibers with an inner diameter in the range of 1–2 μm .

4. Discussion

This study demonstrated that multiple failure patterns occur simultaneously in the annulus fibrosus due to the composite laminate structure, and that alterations in the laminate structure with degeneration (i.e., increase in layer thickness, focal disruption or existing cracks) will increase interlaminar shear stresses and may provide a mechanism for damage in the annulus fibrosus. Lamination theory and additional theoretical analyses were used to calculate stresses in the annulus layers, interlaminar shear stress, and the ineffective length of a broken fiber. SEM was used to evaluate failure patterns in the annulus and novel structural features of the disc tissue.

The annulus fibrosus, and composite materials in general, are effective at resisting failure because the laminated structure of the material resists crack propagation and requires multiple cracks and micro-failure to occur prior to final failure of the laminate while more homogeneous materials typically have a single crack that propagates through the entire material. As a result, failure of the annulus will involve damage initiation, damage accumulation, and final failure. Failure mechanisms in a single layer of a composite include fiber failure and propagation of fiber failure with fiber pull-out from longitudinal tension, matrix cracking from transverse tension, and fiber buckling from longitudinal compression (Fig. 7) (see Swanson, 1997; Herakovich, 1998; Jones, 1999 for reviews). Multiple failure mechanisms may be present within a layer, depending on the history of stresses applied and number of loading cycles. On the laminate level (i.e., multiple layers), these microscopic mechanisms result in transverse cracks in planes parallel to the fibers, fiber-dominated failures in planes perpendicular to the fibers and delaminations between layers of the laminate. Multiple fiber failure is the most dangerous failure mechanism as the fibers are typically the primary load-carrying component, and loss of load carrying ability in one ply may lead to failure of adjacent plies.

Multiple failure patterns including fiber failure, matrix cracking, and delamination were observed in the annulus using SEM with cryo-fracture. Theoretical results indicated that fibers were the dominant load carriage mechanism in the annulus in tension by several orders of magnitude due to the high fiber modulus relative to matrix modulus. When a single fiber in the annulus fails due to localized damage from mechanical or enzymatic means, then the stress must be shared by the adjacent fibers. While the ineffective length in the annulus is fairly large due to the very high values for collagen fiber modulus relative to matrix shear modulus, the stress concentration is still localized to a small region (<approximately 5 μm). If the stress continues to increase or collagen damage is not localized to a small region, then propagation of fiber failure may occur resulting in the failure of a ply. Matrix cracking (corresponding to splitting between the fibers in a single layer) occurs when the strength of

the matrix is exceeded due to transverse tension, yet this does not lead to failure of a laminate. Fiber buckling is due to longitudinal compression in a layer and was not predicted from the uniaxial tension simulation in this study, but would occur under axial compression or complex loading of the annulus and has been described (Vernon-Roberts, 1987; Yu et al., 2003). A new crack from any means will create an associated stress-free edge and a potential new site for biological degradation.

Interlaminar shear stresses and fiber failure were both evaluated quantitatively in this study. The longitudinal stresses in the fiber direction were approximately 8 MPa and somewhat lower than the experimentally determined longitudinal failure stress (i.e., 10.3 MPa (Skaggs et al., 1994)). The interlaminar shear stresses ranged from 0.4 to 1 MPa (Fig. 4), similar to values previously reported (Goel et al., 1995), but interlaminar shear strength is not in the literature. As interlaminar shear stresses are greatest near stress-free edges, focal disruptions or existing cracks may make the annulus more prone to delamination. A computational simulation of the disc degeneration process demonstrated that failure initiated at the end-plates prior to annulus rupture and failure propagation (Natarajan et al., 1994). Goel et al. (1995) used a three-dimensional finite element model of a motion segment with composite annulus to demonstrate that the presence of radial and circumferential tears in the annulus resulted in large increases in interlaminar shear stresses. These investigators also found that for a given axial load on the motion segment, interlaminar shear stresses were higher in the poster-olateral region of the innermost layers of the annulus of an intact disc. This study expanded on those results by demonstrating that a decrease in the number of layers and increase in the thickness of each layer, as reported with degeneration and aging (Marchand and Ahmed, 1990), directly increases the interlaminar shear stresses and is another potential cause for delamination in the degenerated disc. Delaminations were previously reported as failure mechanisms for isolated annulus fibrosus specimens (Iatridis et al., 2003), and separation of layers has been demonstrated in the annulus of intact and denucleated motion segments (Adams and Hutton, 1983; Seroussi et al., 1989; Meakin and Hukins, 2000). As a result, we conclude that delaminations near a focal disruption or existing tears in the annulus are likely implicated in annulus damage associated with circumferential tears while propagation of fiber breaks was considered a likely failure mode associated with radial tears under extreme loading conditions or when collagen damage occurs over a reasonably large region as may occur with biological degradation.

To our knowledge, this is the first study to report the presence of small tubules in the annulus fibrosus parallel to the fibers, and is considered an important finding. These small tubules are similar to those reported in articular cartilage (ap Gwynn et al., 2000), and have significant implications for microstructural modeling. First, the structure of these tubules may contribute to the tensile or compressive modulus. Second, the small tubules, presumably filled with proteoglycans and water, may suggest a mechanism for compartmentalization of water in cartilaginous tissues thereby accounting for differences in total and effective proteoglycan concentrations. Both of these factors may explain in part why the osmotic pressure of proteoglycans in the cartilaginous tissues contributes to only a portion of the compressive modulus (as previously described, e.g., Urban and McMullin, 1988; Lai et al., 1991; Iatridis et al., 1998). Another structural feature noted in SEM analysis was a distinct collagenous structure representative of a pericellular matrix in the nucleus

region. Similar encircling layers of extracellular matrix were previously reported for human disc tissue with transmission electron microscopy (Gruber and Hanley, 2002).

Limitations of this analysis with classic lamination theory include linear elasticity, small strains, simplified rectangular geometry, a single fiber angle, and a lack of compression/tension nonlinearity. Lamination theory assumes no slip between the fibers and matrix, and an average property for fiber and matrix based on their volume fractions that does not distinguish intermolecular interactions in the hierarchical structure of collagen. While the linear elasticity and small strain assumptions may not hold at failure stress and strain levels, it should be noted that the purpose of this paper was to provide insights into failure mechanisms in the annulus fibrosus and not to provide the most rigorously validated constitutive model. Additional complexities of material behaviors, structure, and three-dimensional geometry can be added in future analyses as appropriate. Values obtained for fiber angle were consistent with that found experimentally in inner and middle annulus (Cassidy et al., 1989); however values for G_{12} were only similar to experimental values when determined by curvefitting to axial tension or both circumferential and axial tension experiments (i.e., loading Cases 3 and 4) but higher than experimental values when determined with a curvefit to experimental behavior of circumferential tension alone (i.e., Cases 1 and 2). The variance in material properties reported with testing conditions was anticipated since the anisotropic properties are sensitive to testing orientation and shear stress was not measured in the experiments. These results underscore the requirement that anisotropic material property determination requires data from tests in multiple orientations. A final limitation of this study was our use of rat tail intervertebral disc tissue for SEM analyses. Future studies are necessary to investigate how failure patterns and collagenous structure varies with species and aging. While the laminate structure for rat tail and human lumbar discs shares many similarities, the layer thickness of rat tail annulus fibrosus measured in this study is nearly $10 \times$ lower than reported for human lumbar annulus fibrosus (Cassidy et al., 1989; Marchand and Ahmed, 1990). In the context of these structural differences, we used material properties and structural measurements from human annulus fibrosus for the composite model. The purpose of this SEM analyses then was to demonstrate the presence of certain failure patterns and structural features, and not to quantify dimensions and numbers of structural features.

In conclusion, lamination theory predicted fiber modulus was more than 3 orders of magnitude greater than matrix modulus, values for interlamellar fiber angle and shear modulus were similar to experimental values in the literature, and fiber failure and interlaminar shear stresses may both contribute to annulus failure. Delamination in the annulus was a result of interlaminar shear stresses, was considered a cause of failure in isolated annulus fibrosus tissue (Iatridis et al., 2003), and may be a cause for propagation of circumferential tears in the intact annulus near focal disruptions or existing cracks. Stress concentrations in the annulus due to an isolated fiber break were localized to a region approximately 32 fiber diameters and considered a likely cause of radial tears under extreme loading conditions or when collagen damage occurs over a larger region. SEM analysis of cryo-fracture sites of intervertebral disc tissue demonstrated a clear laminate structure, delamination, matrix cracking, and fiber failure. SEM also detected the presence of small tubules that appear to run along the length of collagen fibers.

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Fig. 1.

(a) Schematic of fiber-reinforced laminate representation of the annulus fibrosus of the intervertebral disc demonstrating axial stress (σ_x) and in-plane shear stress (τ_{xy}). The x - y coordinate system is taken with the structure, i.e., x is in the direction of circumferential hoop stress, and y is in the axial direction. The 1-2 coordinate system is oriented with directions 1 and 2 parallel and perpendicular to the fibers, respectively, with fiber angle θ . (b) Partial free-body diagram demonstrating direction of axial stress, in-plane shear stress, and interlaminar shear stress τ_{xz} , as well as partial delamination, and free surfaces (which are absent of stresses for a uniaxial tension test). (c) Schematic representation of cross section of the eight-layer annulus layup (aspect ratio was modified for clarity).

Fig. 2.

(a) Schematic of a broken collagen fiber with adjacent unbroken fibers. Note that the distance between fibers, h , is taken as the fiber radius. (b) Free-body diagram for a segment of collagen fiber. Results of this analysis indicated that the stress in the broken fiber is back to 95% of its nominal value at 32 fiber diameters away from the break ($\sim 5 \mu\text{m}$).

Fig. 3.

Stresses throughout the thickness (z -direction) on the annulus layers resulting from 10% strain in the circumferential (x -direction) for angle-ply laminate with eight layers and fiber angle $\theta = 39^\circ$. (a) In the global coordinate system stresses are given in the x -direction (σ_x), y -direction (σ_y), and for in-plane shear in the x - y direction (τ_{xy}) (i.e., where x , y , and z corresponds to the circumferential, axial, and radial anatomical directions, respectively). (b) In the material coordinate system, stresses are given in the longitudinal direction (σ_1), transverse direction (σ_2) and for in-plane shear (τ_{12}). The fiber orientation with z -position is superimposed on both graphs.

Fig. 4.

Interlaminar shear stresses for angle-ply laminate of eight-layer angle-ply laminate (circle) and four-layer angle-ply laminate (square) with same total thickness and fiber angles of 39° . The fiber orientation with z -position is superimposed for both the eight-layer simulation (top) and four-layer simulation (bottom). Note that a laminate with fewer layers has a larger magnitude of τ_{xz} than the laminate with thinner layers, and may be responsible for circumferential tears.

Fig. 5. SEM of two different regions of interest in the rat tail intervertebral disc nucleus pulposus demonstrating: (a) fine collagenous structure of the rat nucleus pulposus; (b) cellular inclusion from nucleus pulposus cells demonstrating area where cell was present; and (c) high-magnification image of cellular inclusion demonstrating fine structure of pericellular matrix.

Fig. 6.

SEM of annulus fibrosus of rat tail intervertebral disc demonstrating: (a) angle-ply laminate structure with collagen fibers protruding from the fracture site. An additional view of the laminate structure with delamination and matrix failure is shown under low (b) and high (c) magnification, with (d) high-magnification image of microtubule structure in the collagen network.

Fig. 7.

Schematic representation of typical failure patterns in a fiber-reinforced layer of composite materials. At the laminate level (i.e., multiple layers), these patterns manifest themselves in the form of transverse cracks in planes parallel to the fibers, fiber-dominated failures perpendicular to the fibers, and delaminations.

Table 1

Microstructural and single ply material parameters used for the model

Parameter	Meaning	Value	Source
V_f^a	Fiber volume fraction	0.15	Skaggs et al. (1994)
V_m	Matrix volume fraction	0.85	$V_m = 1 - V_f$
E_f^a	Fiber modulus	904 MPa	$E_f = (E_l - E_m V_m) / V_f$
E_m^a	Matrix modulus	0.5 MPa	Iatridis et al. (1998)
ν_{12}^a	In-plane Poisson's ratio	0.3	Chosen at this value ^b
G_{12}^a	In-plane shear modulus	0.3 MPa	From nonlinear regression
θ^a	Fiber angle	39°	From nonlinear regression
E_l	Longitudinal modulus	136 MPa	Skaggs et al. (1994)
E_2	Transverse modulus	0.76 MPa	Halpin-Tsai equation: $E_2 = E_m(1 + \xi \eta V_f) / (1 - \eta V_f)$ where $\xi = 2$; $\eta = (E_f/E_m - 1) / (E_f/E_m + \xi)$

^aIndependent material constants required for the linearly elastic plane stress analysis.^bParametric study demonstrated insensitivity of in-plane stiffness matrix to this parameter.