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Optical coherence tomographic elastography reveals mesoscale shear strain inhomogeneities in the annulus fibrosus

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STRUCTURED ABSTRACT

Study Design—Basic science study using in vitro tissue testing and imaging to characterize local strains in annulus fibrosus (AF) tissue.

Objective—To characterize mesoscale strain inhomogeneities between lamellar and inter-/translamellar (ITL) matrix compartments during tissue shear loading.

Summary of Background Data—The intervertebral disc (IVD) is characterized by significant heterogeneities in tissue structure and plays a critical role in load distribution and force transmission in the spine. In particular, the AF possesses a lamellar architecture interdigitated by a complex network of extracellular matrix components that form a distinct ITL compartment. Currently, there is not a firm understanding of how the lamellar and ITL matrix coordinately support tissue loading.

Methods—AF tissue samples were prepared from frozen porcine lumbar spines and mounted onto custom fixtures of a materials testing system that incorporates optical coherence tomography (OCT) imaging in order to perform tissue elastography. Tissues were subjected to 20 and 40% nominal shear strain, and OCT images were captured and segmented to identify regions of interest corresponding to lamellar and ITL compartments. Images were analyzed using an optical flow algorithm to quantify local shear strains within each compartment.

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Level of Evidence: N/A

Results—Using histology and OCT, we first verified our ability to visualize and discriminate the ITL matrix from the lamellar matrix in porcine AF tissues. Local AF strains in the ITL compartment (22.0 ± 13.8 , 31.1 ± 16.9 at 20% and 40% applied shear, respectively) were significantly higher than corresponding strains in the surrounding lamellar compartment (12.1 ± 5.6 , 15.3 ± 5.2) for all tissue samples ($p < 0.05$).

Conclusions—Results from this study demonstrate that the lamellar and ITL compartments of the AF distribute strain unevenly during tissue loading. Specifically, shear strain is significantly higher in the ITL matrix, suggesting that these regions may be more susceptible to tissue damage and more mechanobiologically active.

Keywords

interlamellar matrix; translamellar crossbridge; lamellae; annulus fibrosus; mesoscale; mesostructure; optical coherence tomography; elastography; biomechanics; intervertebral disc

INTRODUCTION

Degenerative disc disease (DDD) continues to burden human lives as well as national economies worldwide, but an effective preventative strategy remains elusive.[–] As previously observed, the disorder afflicts the entire intervertebral disc (IVD), but the underlying events appear to occur in localized regions and propagate in overlapping phases, one of which involves annular fissuring and disruption of lamellar structure.[•] Reports from the literature strongly suggest a link between mechanical stress and aging/degeneration.[–] It has been proposed that nerve and vascular ingrowth may be potentiated by a permissive microenvironment within annular fissures, whose origins have recently been postulated to arise from excessive shear generated by stress gradients in the annulus fibrosus (AF).

Considering the structural inhomogeneity of the AF,[–] it is possible that certain regions within the annulus tissue may be more acutely susceptible to these types of stress gradients during IVD loading. One plausible candidate is the proteoglycan- and elastin-rich interlamellar and translamellar cross-bridge (ITL) compartment.[–] Although the precise mechanical properties of ITL matrix have never been directly measured, its composition is very distinct from the well organized regions of lamellar collagen, and changes with age and health of the disc. Aggrecan, versican, and type VI collagen accumulate in the ITL space of ovine neonates, despite being absent in newborns. In humans, total elastin content in discs increases with degeneration grade but decreases in scoliotic IVDs. Unique cell morphologies have also been found in the bovine ITL matrix,[•] suggesting that this compartment is subject to a biophysically dissimilar microenvironment relative to the lamellar matrix.

The distinct matrix composition and organization likely result in a mismatch in properties between lamellar and ITL compartments and may lead to significant differences in mechanical responses during tissue loading, potentially as it pertains to AF tissue damage. Previous studies on interlamellar mechanics seem to support this notion. At the macroscale level, interlamellar lap testing showed that the mechanical connection between lamellae is initially robust, but substantial sliding begins to occur with interlamellar damage. Interestingly, this failed interface was able to continue sustaining forces equal to roughly

50% that of intact over great sliding distances. Recently, using a combinatorial approach of tissue testing and computational modeling, we investigated circumferential shear loading of ovine AF tissue and found that the shear modulus is much higher within lamellae than between lamellae. At the microstructural level, collagen fiber sliding and shear deformation within and across AF lamellae have been observed in bovine IVDs during motion segment bending. The morphology of interlamellar matrix under microtensile loading and intradiscal pressurization also suggest vulnerability to damage.

One hurdle in our understanding of how these complex substructures cooperatively govern AF mechanics during potentially damaging large tissue deformations is that current approaches require either microdissection that strips away many of the *in situ* matrix interactions that exist or allow only surface/near-surface measurements. The goal of this study was to leverage the advantages of optical coherence tomography (OCT) to visualize mesostructural elements (i.e. the ITL matrix) in order to gain a better understanding of how this compartment deforms relative to lamellar matrix during damage-inducing levels of tissue shear loading. We have previously demonstrated the use of OCT in characterizing 3-D morphologies of the ITL network at relatively high resolution. In this study, we show that elastographic analysis can be performed using OCT images obtained before and after loading. Specifically, we observed that under high AF tissue deformations the ITL compartment undergoes much higher shear strain than lamellae, suggestive of matrix damage. These differences could theoretically serve as contributing factors to the initiation of annular clefts and fissures, as well as to the stimulation of active tissue remodeling.

MATERIALS AND METHODS

OCT imaging

A swept-source OCT system (Thorlabs, Newton, NJ) was used in this study. Its center wavelength was at 1.31 μm , and the full-width-half maximum (FWHM) bandwidth was 0.1 μm , yielding a theoretical dispersion-free axial resolution of $7.6 \mu\text{m} (= \frac{2 \ln 2}{\pi} * \frac{1.31^2}{0.1})$ in air, or 5.6 μm in IVD where index of refraction $n=1.35$ was assumed. The objective in the OCT sample arm was 10x (NA=0.1; Olympus, Waltham, MA), achieving a theoretical lateral resolution of $\sim 4.8 (= 0.37 * \frac{1.31}{0.1}) \mu\text{m}$.

Each A-scan of the OCT mapped 2.55mm penetration depth in air (or 1.89mm in tissue) in 512 pixels, and the OCT operated at a sweep rate of 16 kHz (16k A-scans per second), scanning $3.6 \times 3.6 \text{ mm}^2$ wide. Therefore, a data cube of $512 \times 512 \times 512$ pixels captured a $3.6 \times 3.6 \times 1.89 \text{ mm}$ field of view (FOV) in about 16.3 seconds. The effective penetration depth was, however, only about 0.5 mm (compared to 1.89mm), which likely results from the scattering nature of the dense collagen in the AF and OCT sensitivity. The OCT delivered 6.3 mW (averaged over the swept spectra) to the sample. By using the adjustable neutral densities as the sample and collecting the reflected light, the sensitivity (signal-to-noise ratio, SNR) was measured being 95 dB.

Histological validation of translamellar cross-bridge imaging by OCT

Skeletally mature porcine lumbar motion segments were generously provided by Dr. Thomas Caperna (United States Department of Agriculture). AF samples were excised from the anterior region and fixed in a 10% neutral buffered formalin solution for 7 days. AF samples were first imaged by OCT. The same samples were then processed in graded ethanol and xylene baths before paraffin embedding (TP1020/EG1160; Leica Microsystems, Buffalo Grove, IL, USA). Paraffin blocks were cut with a microtome (HM355; Microm/Thermo Fisher Scientific, Waltham, MA, USA) to obtain 10 μm thick transverse sections of the AF. Sections were stained with Safranin-O/Fast green and examined at 400x magnification under brightfield and polarized light microscopy. At precise locations of tissue samples, histology images were compared with OCT images to identify corresponding features between imaging modalities.

AF tissue preparation and mechanical shear test combined with OCT imaging

Approximately $1\text{cm} \times 1\text{cm} \times 1\text{cm}$ blocks of porcine AF tissue ($n = 6$) were cut from the anterior region of the IVD for shear tests (Figure 1A). A custom-designed shear testing fixture was combined with a Bose-Electroforce material testing system (Testbench LM-1, Eden Prairie, MN) for shear tests (Figure 1B,C). In order to secure the specimen between the top load and bottom fixed plates, a small amount of Vetbond tissue adhesive (3MTM, Maplewood, MN) was applied to the two plates. AF samples were hydrated with phosphate buffered saline (PBS) and axially directed displacements of 0.20cm and 0.40cm (corresponding to 0.20 and 0.40 engineering shear strain) were applied to load AF tissues in simple shear, at strain rates of 0.01 and 0.02/s, respectively. We selected this range of tissue shear strain, which is higher than what the AF experiences typically during physiologic loading, in order to induce shear-generated ITL matrix damage that was observed in other studies. A swept-source (SS) OCT (Thorlabs) was incorporated into the test setup for visualizing tissue deformation and detecting any sliding of tissue along the fixture plate during shear load (Figure 1B).

Image data analysis

An optical flow technique was used for measuring the AF tissue strain field including the ITL network. Our purpose was to establish an objective, systematic, and automated approach to facilitate the computation of strain field. Optical flow (or image velocity) computes 2-D motion from spatiotemporal patterns of image intensity. The strain field is subsequently derived as the composition of gradient of optical flow. The reason we chose the optical flow approach is because it features an integrated median filtering step into the classical Horn-Schunck model, which assumes similar brightness between images and smooth evolution of the flow field. Previous studies have validated the use of median filtering on raw OCT images,⁷ and we verified that the assumptions of brightness constancy and smoothness were appropriate for our images.

Once the optical field ($U(x,y)$ for the horizontal field and $V(x,y)$ for the vertical field) was estimated, strain was derived as

$$strain(x, y) = \frac{1}{2} \left(\frac{dU}{dy} + \frac{dV}{dx} + \frac{dU}{dx} \frac{dU}{dy} + \frac{dV}{dx} \frac{dV}{dy} \right)$$

To validate the technique, *en face* cross-sectional OCT images of IVD were virtually distorted up to 30% through a known affine transformation. Therefore, the estimated flow field can be compared with the pre-determined field, where 30% indicates the ratio of horizontal shear displacement to the thickness of sample. This technique generated flow fields comparable to the applied 30% virtual shear, a range that sufficiently covers the 10% shear strain intervals that we applied to the AF tissue samples in our real tests.

Elastography using OCT

We sequentially applied 0, 20, and 40% shear strain to IVD. One common optical section of the *en face* cross-sectional OCT data cube was selected (Figure 2A). Based on distinct ITL and lamellar features, regions of interest (ROI) were selected. We intentionally defined ROI near the center of images (Figure 2B), away from the boundaries of the tissue, in order to avoid edge effects due to tissue incision and/or tissue adhesive application. The optical flow technique was applied to each consecutive pair of images. The field between non-consecutive images can be determined by summing fields of consecutive images (i.e., $U_{14} = U_{12} + U_{23} + U_{34}$). Briefly, within each ROI, images were segmented to identify areas corresponding to ITL and lamellar compartments (Figure 3A), and displacement vectors were obtained (Figure 3B). Lagrangian shear strains were computed for pixels within each compartment (Figure 3C, D), and values within each compartment were averaged to obtain one data point for each test. Six (n=6) independent tests/measurements were conducted using different tissue specimens from different animals.

Statistical analysis

All data are expressed as the mean $\pm$ standard error of the mean (SEM). ITL strains were compared to the surrounding lamellar strain using paired t-tests (SYSTAT 12.0). The level of significance was set at $\alpha = 0.05$. Results are represented as means $\pm$ standard deviations.

RESULTS

Histological verification for OCT translamellar cross-bridge images

Structural features that were identified as translamellar cross-bridges in planar optical slices of the volumetric OCT images of AF specimens (Figure 4A) were then located on Safranin-O/Fast green histologically stained paraffin sections of the same specimens viewed under brightfield (Figure 4B) and polarized light microscopy (Figure 4C). Together with our previous results in ovine IVDs, these data indicate that the ability for OCT to visualize the ITL matrix in AF tissues across species.

OCT elastography during mechanical shear loading

To determine the relative deformations of the lamellar and ITL compartments within AF tissues under mechanical shear loading, 3-D volumetric OCT images were captured before loading and at incremental shear deformations. At 0.20 applied shear, the shear component

of the 2-D strain field was of similar value in the ITL matrix (0.220 ± 0.056). However, the shear strain component in the lamellar matrix was significantly lower (0.121 ± 0.023 ; $p=0.028$), slightly more than half of that experienced by the ITL matrix. At 0.40 applied shear, the shear strains of both lamellar (0.153 ± 0.021) and ITL compartments (0.311 ± 0.067) remained significantly different from each other ($p=0.027$), with the lamellar strain roughly half that of the ITL strain. In both compartments, strain did not increase commensurately with the applied strain, indicating a non-linear relationship (Figure 5).

DISCUSSION

Inhomogeneity in extracellular matrix structure and composition contribute to intricate strain patterns within the AF tissue at all levels of scale (Figure 6). With regard to the macroscale level, both geometric parameters and material properties vary significantly in the radial direction from one lamella to the next, though less so circumferentially along lamellae. Within each lamella at the microscale level, it has been shown that motion segment bending induces fibrillar sliding of collagen on the convex side that is in tension, resulting in greater relative displacements across collagen fibers than within collagen fibers, similar to that which has been observed in tendons.⁷ At the intermediate mesoscale level, the ITL matrix traverses through and between lamellae, but its mechanical function in the AF during tissue loading is still subject to speculation. Previous studies have suggested that the ITL compartment contributes little to AF mechanics in radial tension.²⁹ Instead, it has been postulated that the ITL may play a greater role in maintaining annular integrity during shear loading,⁷ as supported by the absence of sliding between lamellae due to fibrous interlamellar connectivity. At high deformations, however, the ability for interlamellar sliding to occur suggests complexity in the load sharing between the ITL and lamellar matrix. To our knowledge no explicit measurements of the deformations within these compartments have been simultaneously quantified. Elucidation of the internal deformations within the AF is important for understanding how the ITL and lamellar compartments coordinately distribute strain during tissue loading. Our recent work using OCT to visualize the ITL matrix has enabled this current approach to characterize deformations at depths of 1–2mm from the cut surfaces of AF tissues.

Results from this present study indicate loaded AF tissues experience shear strains in the ITL matrix that are approximately twice those measured within the lamellar matrix. Furthermore, changes in both ITL and lamellar strain decreased with greater applied load. These observed differences are qualitatively consistent with trends we found in a previous study, in which a nonlinear constitutive model parameters fit to surface strain measurements yielded a interlamellar shear modulus that was roughly half the lamellar shear modulus, both of which increased with higher applied strain (strain stiffening). However, in contrast to previous studies using lower applied shear strains, our averaged ITL and lamellar shear strain was not equal to the applied strain; this could be an indication of matrix damage, likely in the ITL compartment. Our data also corroborates microscale measurements that greater strain is induced between interlamellar cells, relative to lamellar cells, during biaxial testing of bovine AF tissues.

Although there are currently no validated theories that explicitly relate tissue stress/strain to AF damage, it is possible that the differential ITL and lamellar matrix properties could potentially contribute to what has been observed in disc injury or degeneration. Delamination between annular lamellae, which plays a role in the progression of disc herniation, has been postulated to occur by excessive shear.²⁷ Likewise, shear has been implicated in the formation and propagation of annular fissures that are hallmarks of disc degeneration. If the ITL matrix does play an important functional role, it would suggest that the distribution of translamellar cross-bridges in the AF might correspond with regions of susceptibility to injury. For instance, one could speculate that the presence of translamellar cross-bridges might serve as regions which can better withstand shear deformations, to buffer annular lamellae under large tissue deformations. With smaller cross-bridge densities in the posterior AF, the greater shear demands on existing cross-bridges could be one reason for enhanced susceptibility of the posterior AF to injury under various modes of disc loading.²⁸ Further studies mapping age- and degeneration-related changes in ITL morphology, and investigation of failure mechanisms of the AF tissue would further illuminate this relationship.

The mismatch in properties and function between the ITL and lamellar compartments is also likely to have significant effects on cellular mechanoregulation and, hence, tissue health, given the interdependence between the extracellular matrix and resident cells. Higher shear strain in the ITL could imply enhanced mechanical stimuli. The distinct micromechanical environments could additionally explain morphological differences that have been observed between cells of the ITL and lamellar compartments. However, the precise effects of differential shear stimuli on AF cells is not yet known. Alterations in properties of one compartment of the AF could also modify the distribution of strain between the compartments, potentially triggering cell-mediated tissue remodeling and leading to long-term structural changes throughout the AF matrix.

An advantage of using OCT for laser-based imaging is its penetration depth in collagenous tissues, which enabled measurement of *in situ* deformations of both the ITL and lamellar matrix internal to the AF during loading. Greater imaging depth during elastography helps diminish any effects of structural damage due to incisions made during specimen preparation. Our tissue samples were imaged 1–2mm under the transverse plane of the AF, and we sought to decrease any artifacts further by selecting ROI several millimeters away from the radial and circumferential cut faces. Loading of tissue samples in simple shear by specifying displacements at the radial surfaces is a potential limitation of the experimental approach, because it does not completely represent physiological conditions. Shear deformations in the AF can be induced with annular bulging during IVD compressive loading that results in biaxial tension in the circumferential and axial directions. *In situ* experimental tests of intact IVDs under various physiologic spinal motions are needed to determine the full implications of differential deformations between ITL and lamellar compartments.

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Figure 1. Experimental setup for this study

A: AF tissue preparation for OCT imaging. Specimens were cut from the anterior region of lumbar discs with radial and axial dimensions of 7–9mm and a circumferential length of 10–12mm. B: Materials testing system modified with custom fixtures for shear strain application and outfitted with OCT scanner. C: Illustration of the area marked with the dashed line in B. Tissue specimens were affixed to custom fixtures and a circumferentially directed force was applied to induce simple shear deformation. Imaging was performed on the transverse (radial-circumferential) cut face of the tissue.

Figure 2. Representative OCT images of a tissue specimen before (left) and after (right) undergoing simple shear deformations

A: Image of the entire tissue sample used for the test. B: Region of interest where strains were calculated. In order to avoid edge effects of both tissue incisions made during specimen preparation and tissue adhesive used to affix tissues to the plate fixtures, ROI were selected to be several millimeters away from the top and bottom plates and radial boundaries, as indicated by the yellow box in A.

Figure 3. Elastographic analysis of OCT images

A: Superimposed pseudocolored OCT images of ROI before (red) and after (green) application of 20% nominal shear strain. B: These OCT images were used for optical flow analysis to obtain an array of displacement vectors. C: 2-D Lagrangian shear strain intensity map computed using displacement gradients in the image plane. Note the high values of shear strain in the areas corresponding to ITL compartment. D: Surface plot representation of the shear strain data shown in panel C.

Figure 4. Verification of OCT image segmentation of the ITL compartment

A: A transverse optical section (radial-circumferential plane) from the 3-D OCT image volume of the intact tissue specimen. Lamellar matrix, marked by the letters “a” and “b,” is dark on OCT images. Translamellar crossbridges (arrows) and interlamellar matrix (asterisks) have significantly higher intensities. B: The same tissue specimen was marked, processed, paraffin sectioned, and then stained with Safranin-O/Fast green. Light micrograph of the corresponding area in panel A reveals matching features as those found in OCT images, confirming accurate identification of the ITL compartment. C: The same tissue section as in panel B, imaged under polarized light, enhancing contrast of the different layers of annular lamellae. Scale bar = 1000 μm .

Figure 5. Comparison of average compartmental shear strains

Average shear strain (n=6) measured in the ITL compartment was significantly higher than that in the lamellar compartment for both 20% (* $p=0.028$) and 40% (** $p=0.027$) nominal applied shear strain. Moreover, trends indicate a non-linear relationship between applied nominal shear strain and the local Lagrangian shear strain induced. Data are shown as mean $\pm$ SEM.

Figure 6. Schematic diagram illustrating the complex determinants of local matrix deformation in AF tissue

The lamellar architecture of the AF imparts macroscale inhomogeneities in matrix strain. At the mesoscale, the distinct properties of the ITL compartment and complex structural network of translamellar crossbridges complicate load distribution. Collagen composition and organization contribute to inhomogeneity at the microscale.