Published in final edited form as:

Biomech Model Mechanobiol. 2017 August ; 16(4): 1425–1438. doi:10.1007/s10237-017-0896-4.

Image-based multiscale mechanical modeling shows the importance of structural heterogeneity in the human lumbar facet capsular ligament

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Abstract

The lumbar facet capsular ligament (FCL) primarily consists of aligned type I collagen fibers that are mainly oriented across the joint. The aim of this study was to characterize and incorporate in-plane local fiber structure into a multiscale finite element model to predict the mechanical response of the FCL during in vitro mechanical tests, accounting for the heterogeneity in different scales. Characterization was accomplished by using entire-domain polarization-sensitive optical coherence tomography to measure the fiber structure of cadaveric lumbar FCLs ($n = 6$). Our imaging results showed that fibers in the lumbar FCL have a highly heterogeneous distribution and are neither isotropic nor completely aligned. The averaged fiber orientation was $+9.3^\circ$ ($+29.9^\circ$ in the inferior region and $+5.1^\circ$ in the middle and superior regions), with respect to lateral–medial direction (superior–medial to inferior–lateral). These imaging data were used to construct heterogeneous structural models, which were then used to predict experimental gross force–strain behavior and the strain distribution during equibiaxial and strip biaxial tests. For equibiaxial loading, the structural model fit the experimental data well but underestimated the lateral–medial forces by $\sim 16\%$ on average. We also observed pronounced heterogeneity in the strain field, with stretch ratios for different elements along the lateral–medial axis of sample typically ranging from about 0.95 to 1.25 during a 12% strip biaxial stretch in the lateral–medial direction. This work highlights the multiscale structural and mechanical heterogeneity of the lumbar FCL, which is significant both in terms of injury prediction and microstructural constituents' (e.g., neurons) behavior.

Keywords

Fiber structure; Collagen fibers; Fiber imaging; Structural heterogeneity; Multiscale modeling; Facet capsular ligament; Spine

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

1 Introduction

The lumbar facet capsule is a fibrous soft tissue connecting the posterior articular facets of adjacent vertebrae. The capsule surrounds the facet joint space and consists of two layers (Yamashita et al. 1996): the ventral ligamentum flavum and the dorsal facet capsular ligament (FCL). The lumbar FCL has a complicated structure, comprising a hydrated ground matrix of various biomacromolecules within which stiff type I collagen fibers reside (Yamashita et al. 1996). Heterogeneity, anisotropy, nonlinearity, and the ability of fibers to rotate and reorient under loading all complicate the mechanical behavior of the FCL, at both the macroscopic and the microscopic scale. Macroscopically, the structure of the tissue determines how gross loads or joint motion lead to strain in the tissue. Microscopically, the fibers transmit force and strain to the biologically active constituents, such as nociceptive nerve fibers (McLain and Pickar 1998) and fibroblasts that may be responsible for tissue remodeling (Yahia and Garzon 1993).

To explore the complex multiscale mechanical behavior of the lumbar FCL, a multiscale theoretical approach is mandated. Structural soft tissue models, which can incorporate the tissue architecture in a novel way, have been widely used in various forms (Lanir 1983; Holzapfel et al. 2000; Gasser et al. 2006; Stylianopoulos and Barocas 2007). These structure-based models are appealing, but require specification of fiber alignment. As shown in the scanning electron microscopy (SEM) images of Fig. 1, even within the same FCL, there are profound variations in the direction and degree of fiber orientation. The impact of this variability is not well understood and is thus a key issue in lumbar FCL mechanics.

Although SEM provides compelling evidence of structural heterogeneity and anisotropy, it can only image very small sections of the tissue and thus is also not feasible for full-tissue characterization. Polarized light imaging (Tower et al. 2002), as used previously for cervical FCL (Quinn and Winkelstein 2010), is not feasible because of the lumbar FCL's opacity. The optical anisotropy (birefringence) exploited by polarized light imaging originates from the alignment of macromolecular arrays within the tissue, and is still present in the lumbar FCL but not measurable by a transmission-based modality. Polarization-sensitive OCT (PS-OCT) (De Boer et al. 1997), however, can provide retardance and optic axis orientation contrasts that originate from tissue anisotropy (birefringence), in a noninvasive reflectance-based image. PS-OCT has been used previously to study the structure of various birefringent biological tissues, including bone (De Boer et al. 1999), brain (Wang et al. 2011, 2014; Liu et al. 2017), and lumbar FCL (Claeson et al. 2015). The collagen fiber organization in the ligament can be assessed by retardance and optic axis orientation contrast from PS-OCT.

This project pursued two sequential objectives. First, the entire-domain collagen fiber organization in the lumbar FCL was characterized by PS-OCT. Second, macroscale FCL behavior was compared to in vitro experiments through construction of subject-specific multiscale finite element models based on the structural data. Collectively, these two objectives allowed us to test the hypothesis that structural heterogeneity is important in determining lumbar FCL mechanical behavior, where it leads to multiscale heterogeneity in the mechanical response.

2 Materials and methods

2.1 Acquisition of mechanical test data

Planar biaxial test data from a previous study (Claeson and Barocas 2017) were used; for details of the mechanical tests, the reader is referred to Claeson and Barocas (2017), but the essential elements of that study are summarized briefly here. Six healthy L4–L5 cadaveric lumbar FCLs (right $n = 2$, left $n = 4$) from four motion segments (ages 35–65 years) were cleared of the surrounding musculature and tissues, flattened, trimmed to a cruciform shape, and mounted on an Instron-Sacks planar biaxial tester along the bone-ligament-bone axis (lateral–medial, L–M) and the perpendicular axis (superior–inferior, S–I). Samples were clamped and pulled to a preload of ~ 1 N on all four sides to remove any slackness in the tissue. The tissue surface was speckled with Verhoeff's stain to enable displacement tracking by digital image correlation (Raghupathy et al. 2011). The testing protocol included ten cycles of preconditioning to 12% grip strain at a rate of 3 mm/s, followed by all combinations of one-, two-, three-, and four-arm extensions (Raghupathy et al. 2011) and concluded by an equibiaxial extension at the same grip strain and rate. A grip strain of 12% was chosen such that the tissue nonlinear behavior could be studied without damaging the sample. The grip displacement rate was chosen such that the viscoelastic effects were minimized.

2.2 PS-OCT imaging and image processing

A polarization-maintaining fiber (PMF)-based spectral domain PS-OCT system was used to probe the microstructure of the samples by quantifying the phase retardance, and in-plane optic axis orientation. Detailed system descriptions have been reported previously (Wang et al. 2010; Liu et al. 2016). A broadband super luminescent diode centered at 840 nm with a 50 nm b and width was used as light source, yielding an axial (depth, z) resolution of $5.5\mu\text{m}$ in the tissue (refractive index: ~ 1.4). A scan lens in the sample arm ensured a large field of view with a lateral resolution of $\sim 15\mu\text{m}$ (xy). The PMF introduces an arbitrary offset to the optic axis orientation, which is sensitive to environmental factors (fiber movement and temperature change). In order to get the correct optic axis orientation, a dynamic calibration method was used (Liu et al. 2016). The system's signal output was used to calculate reflectivity, phase retardance and in-plane optic axis orientation. The phase retardance $\mathcal{R}(z)$ quantified the strength of alignment (degree of anisotropy) of fibers, and the in-plane optic axis orientation $\mathcal{A}(z)$ was used to determine the in-plane collagen fiber orientation.

Prior to the PS-OCT imaging experiment, each sample was clamped biaxially and loaded with a ~ 1 N force on each side to keep it as flat as possible. The 1 N force was chosen to match the preload applied on each grip during the biaxial mechanical tests. Due to the large size of the sample and uneven sample surface, each sample was imaged by a tile scan.

Histogram- and integration-based approaches, similar to those described in (Wang et al. 2014), were used to obtain the *en-face* fiber orientation and phase retardance maps for each 3D dataset, respectively. For each A-line (through-thickness profile of imaging pixels), a depth of 30 pixels (0.132mm) for the retardance and fiber orientation profiles was considered. The *en-face* values of retardance were calculated by taking the mean of

retardance profile, whereas *en-face* values of fiber orientation were found by selecting the angle with the highest probability distribution over each A-line.

The *en-face* maps were stitched to form in-plane fiber orientation and retardance maps over the whole sample. These maps were manually registered to photograph of the sample geometry to adjust their boundaries to those of the sample at the onset of biaxial test. The overall fiber orientation and retardance *en-face* maps were filtered by applying average and median filters, respectively. Fiber orientation map filtering was performed on the corresponding orientation tensor map.

2.3 Construction of a global orientation tensor to find average fiber orientation

The concept of the orientation tensor (i.e., the second-order fabric tensor; Cowin 1985) is critical when dealing with fiber orientation. The orientation tensor converts fiber orientation, which is not additive, to a quantity to which the principle of superposition and other linear operations can be applied. Any operation (e.g., filtering, averaging, coarsening) on the fiber orientation fields was performed on the orientation tensor. For each imaging pixel's measured angle $\theta^{(p)}$ (as shown in Fig. 3), the orientation tensor associated with that pixel is defined as follows:

$$T^{(p)} = \begin{bmatrix} \cos^2 \theta^{(p)} & \sin \theta^{(p)} \cos \theta^{(p)} \\ \sin \theta^{(p)} \cos \theta^{(p)} & \sin^2 \theta^{(p)} \end{bmatrix} \quad (1)$$

Note that $\theta^{(p)}$ is measured with respect to the X axis (L–M direction). Since the trace of T is $\cos^2 \theta + \sin^2 \theta = 1$, by construction, the eigenvalues of T must sum to unity. The primary fiber direction is the eigenvector of T corresponding to its larger eigenvalue, and the difference between the two eigenvalues represents the degree of anisotropy in the primary direction.

Separately, for each sample, the average fiber orientation was calculated through retardance-weighted averaging of the orientation tensors, such that:

$$T^{(\text{sample})} = \frac{1}{1 + \sum_1^N \delta^{(\text{A-line})}} \left(\frac{1}{2} I + \sum_1^N \delta^{(\text{A-line})} T^{(\text{A-line})} \right) \quad (2)$$

where $T^{(\text{sample})}$, $\delta^{(\text{A-line})}$, N , and I denote the sample's average orientation tensor, each A-line's retardance, number of A-lines belong to that sample, and identity tensor, respectively. The orientation tensor for each A-line was weighted by its retardance to account for each angle's relative contribution in averaging. Thus, if at one A-line the tissue was highly aligned (large δ), the direction associated with that A-line contributed strongly to the average, but if the tissue was nearly isotropic (small δ), a nearly isotropic orientation tensor ($\sim \frac{1}{2} I$) resulted.

The overall sample retardance was considered proportional to the difference between the eigenvalues of its average orientation tensor (Chandran and Barocas 2007). If (v_1, v_2) and (λ_1, λ_2) are orientation tensor's eigenvectors and eigenvalues, such that $\lambda_1 > \lambda_2$, then:

$$\delta \propto \lambda_1 - \lambda_2 \quad (3)$$

The rationale to use the difference between the eigenvalues of the averaged orientation tensor, not the average value of constituent A-lines' retardance, for the overall sample retardance, was that a wide range of different orientations over a sample suggests low average anisotropy, that is a low retardance, but averaging the local retardance values does not necessarily produce this result. Averaging the individual fiber orientation tensors for the different fiber orientations over each sample, however, leads to a more isotropic average orientation tensor, and consequently a lower (and thus more representative) difference between the eigenvalues. Examples to illustrate this concept are presented and discussed in the "Appendix 1."

Each sample's anisotropy (α) was measured as the difference between the eigenvalues of the overall orientation tensor, such that:

$$\alpha = \lambda_1 - \lambda_2 \quad (4)$$

A completely isotropic sample has α value of 0, and a completely aligned sample (in any direction) has α of 1.

To compare with previous observations (Yamashita et al. 1996), the average fiber orientation among samples was calculated. First, the FCL's geometry from the right facet joint was mirrored about the superior-inferior axis to match its anatomical sites to those of the left FCLs. Next, each sample was divided into three equally spaced regions in the S-I direction (inferior, middle, and superior regions). Then, for each region, the data for all samples were pooled and, by the same method as Eq. (2), the average orientation tensor was calculated. Once the orientation tensors had been obtained, the primary eigenvectors were used to determine the fiber orientation. This method was used to calculate the overall average fiber orientation (θ^{ot}), the average fiber orientation in the inferior region (θ^{inf}), and the average fiber orientation in the middle and superior regions ($\theta^{Mid-Sup}$).

2.4 Image-based multiscale method

A multiscale finite element method (Chandran and Barocas 2007; Stylianopoulos and Barocas 2007) was used to relate the structural information (local fiber orientation and degree of anisotropy) to the tissue's overall mechanical behavior. The multiscale method connects two scales (Fig. 2): the *macroscale*, representing the tissue continuum level, and the *microscale*, consisting of representative volume elements (RVEs) embedded within each macroscale element. Each RVE contained a computational network of ~ 400 fibers oriented in a certain direction along with a parallel neo-Hookean matrix.

Detailed descriptions of this method along with mathematical formulation are explained in previous studies (Chandran and Barocas 2007; Stylianopoulos and Barocas 2007), but we describe it here briefly. More details of the formulation are given in “Appendix 2.” In the multiscale scheme, unlike single-scale finite element methods, which use a constitutive equation to obtain deformation-based macroscale stresses, macroscale stresses are determined by the deformation of the underlying RVEs, each of which deforms based on the displacement of the surrounding macroscale finite element. Deformed RVEs generate stress in the fiber network (obtained by averaging over the RVE volume) and the neo-Hookean matrix, which together form the total RVE stress. The coupled process iterates between the two scales until equilibrium is achieved. Fibers follow the phenomenological constitutive equation (Billiar and Sacks 2000):

$$F=A [\exp (BE)-1] \quad (5)$$

where F and E are force and Green strain for fibers, and A and B are two constitutive constants. The neo-Hookean part of RVEs, modeling the non-fibrillar components of the tissue, was considered to have low compressibility (Poisson’s ratio $\nu=0.45$).

Each FCL’s geometry was constructed and meshed with linear hexahedral elements using Abaqus CAE based on the initial, undeformed image of a biaxial test. Since the finite element grid was much coarser than the OCT resolution, a proper averaging method, similar to that used to obtain *en-face* orientation fields, was required to find the fiber orientation of each finite element. After laying the coarse grid of finite elements over the fine grid of OCTA-lines, we obtained the elemental fiber orientation by averaging the fiber orientation tensor of its constituent A-lines, such that:

$$T^{(\text{finite element})}=\frac{1}{1+\sum_1^N \delta^{(\text{A-line})}}\left(\frac{1}{2}I+\sum_1^N \delta^{(\text{A-line})}T^{(\text{A-line})}\right) \quad (6)$$

where $T^{(\text{finite element})}$, $\delta^{(\text{A-line})}$, N , and I denote elemental orientation tensor, each A-line’s retardance, number of A-lines belong to each element, and identity tensor, respectively. Subsequently, each element’s average fiber orientation was calculated using its orientation tensor’s eigenvalues and eigenvectors. As above, the elemental retardance was considered proportional to the difference between the two eigenvalues of the elemental orientation tensor. An example of the average procedure and its results are given in “Appendix 3.”

An RVE containing a fiber network was constructed at each Gauss integration point in each element according to its fiber orientation and retardance. First, an isotropic Delaunay network was created based on random 3-D seed points. Next, the network was stretched geometrically based on the retardance and rotated to align in the elemental fiber direction. The final network was then cropped to a cubic subsection for use as the RVE.

2.5 Simulation and data analysis

The macroscale domain of the simulations was deformed based on optical displacement tracking of tissue boundaries during the experiments. Tracked displacements were smoothed to reduce noise, and each boundary was displaced according to the displacement of its midpoint to avoid abrupt large stresses on the boundary elements in the simulations. The same model was used to simulate the FCL's mechanical response to equibiaxial extension, to strip biaxial extension (2-arm pull) in the joint direction (L–M), and to strip biaxial extension orthogonal to the joint direction (S–I). Because all structural information was obtained from PS-OCT, only three fitting parameters existed in the models: A (fiber stiffness), B (fiber nonlinearity parameter), and G_{mat} (neo-Hookean matrix shear modulus). These parameters, as well as fiber density and fiber diameter, were assumed to be the same throughout each sample.

For each sample, mesh convergence study has been conducted to verify that the model results were independent further finite element mesh refinement. For each simulation, boundary forces were calculated and plotted with respect to grip strains for all arms and were averaged to obtain the force–strain graph for each direction (L–M and S–I). These results were compared to the experimental results to find the best fitting parameters for each sample separately. Green strain fields were also calculated and compared to the experimental results from digital image correlation (Raghupathy et al. 2011).

Once the models are compared with the experimental results, macroscale and microscale strain-based measurements were calculated throughout each sample to assess the heterogeneity in tissue behavior. For this purpose, strip biaxial tests in L–M direction were chosen. The strip biaxial test in L–M direction was chosen for two reasons: First, it provides a simple loading condition (nearly uniaxial) which better probes the impact of structural heterogeneity on the response by minimizing other potential factors. Second, it is more similar to the in vivo loading conditions during spinal torsion or lateral bending. To show the heterogeneity spectrum, the probability distribution was plotted for each measurement.

Microscopically, each individual fiber's stretch ratio (λ_{fibers})—within microscale RVEs—was calculated by finding its deformed length and dividing by the initial length. Macroscopically, the maximum principal Cauchy stress, maximum principal Green strain, stretch ratio along the L–M direction (λ_{xx}), and averaged elemental fiber stretch ratio ($\langle \lambda_{\text{fiber}} \rangle^e$) were calculated. λ_{xx} was calculated using the right Cauchy–Green tensor's (C) invariant defined as:

$$\lambda_{xx} = \sqrt{a_0 \cdot C \cdot a_0} \quad (7)$$

where a_0 is a unit vector pointing in the L–M direction. The arithmetic average of fiber stretch ratios within each element was used to find $\langle \lambda_{\text{fiber}} \rangle^e$.

3 Results

3.1 Fiber imaging

Although fibers are primarily oriented in the lateral–medial (bone-to-bone) direction, there was considerable heterogeneity over the ligament. This heterogeneity can be observed through the variety of fiber orientations as well as the degree of anisotropy over different regions. Fiber orientation distributions (Fig. 3a) for all six samples showed the wide range of fiber orientation angles, such that L–M was the predominant fiber direction but fiber alignment was measured in many different directions, and showed considerable sample-to-sample variation. Fiber orientation vector plots for two representative samples are shown in Fig. 3b. Each vector's length is proportional to its local retardance. The orientation vector plots for the two representative samples are pointed to their corresponding orientation distributions.

The sample anisotropy suggests that the fibers in our FCL samples were neither isotropic ($\alpha = 0$) nor perfectly aligned ($\alpha = 1$). Overall, sample anisotropy, measured as the difference between the eigenvalues of the overall sample orientation tensor, was 0.55 ± 0.28 (mean $\pm$ 95% CI).

The average fiber orientation in the middle and superior regions ($\theta^{\text{Mid-Sup}}$), among all samples based on the orientation tensor method, was $+5.1^\circ$, with respect to the L–M direction. That is, in these regions, the fiber direction tends to run very slightly from the inferior to the superior as one moves along a fiber from the lateral to the medial boundaries of the tissue. The average fiber orientation in the inferior region (θ_{inf}) was $+29.9^\circ$, with respect to the L–M direction, which shows that the fibers in this region run in the inferior–lateral to superior–medial direction. Overall, the average fiber orientation (θ_{tot}) was calculated as $+9.3^\circ$, with respect to the L–M direction. These results are in agreement with qualitative observations by Yamashita et al. (1996). In their paper, based on macroscopic and microscopic observations, they reported that in the superior and middle regions, fibers orient in a nearly L–M direction. In the inferior regions, however, they observed that fibers run in the superior–medial to inferior–lateral direction.

3.2 Comparison between multiscale simulations and experiments

Average grip forces in both directions (L–M and S–I) for three tests (equibiaxial, strip biaxial in L–M direction, and strip biaxial in S–I direction) are shown in Fig. 4a–c) for the experiment and the model. The error bars indicate the 95% confidence interval (CI) of the experiments. Force–strain graphs show the nonlinear, anisotropic behavior of the FCL. Forces in the L–M direction were significantly higher than the S–I direction ($p < 0.05$), consistent with the primary orientation of fibers being in the L–M direction. Equibiaxial extension tests resulted in larger forces for both directions compared to the strip biaxial tests because of simultaneous pull on all four sides.

The model was able to fit the data well for four of the samples, but it showed poorer agreement for two of the samples. The root-mean-square (RMS) errors for the model were 2.15, 2.11, and 0.68N for equibiaxial, strip biaxial in L–M direction, and strip biaxial in S–I direction, respectively. Best-fit parameters A , B , and G_{mat} for the model are shown in Fig.

4d. For each sample, one set of parameters was used to fit the equibiaxial, strip biaxial in L–M direction, and strip biaxial in S–I direction experiments. These values, averaged over the samples, were $A = 13.4 \pm 8.8 \mu\text{N}$ (mean $\pm$ 95% CI), $B = 20.7 \pm 4.9$, and $G_{\text{mat}} = 37.3 \pm 22.2$ kPa, respectively. Except for the fiber nonlinearity parameter B , the fitting parameters encompass a relatively wide range among different samples. The variability of fiber stiffness parameter A , which is dependent on fiber cross-sectional area, is possibly explained by the variability of collagen fiber diameters in the lumbar FCL samples.

The experimentally measured Green strain field was compared to the results of the model. As can be seen in Fig. 5, the model's strain distribution was visually similar to the experimental results with a high level of strain localization. Figure 5 shows the horizontal (xx) and vertical (yy) components of the Green strain field during equibiaxial extension for two representative samples. Vectors represent the nodal displacements. For both of these representative samples, the experimental data showed high strain at certain locations for each sample. The pooled RMS errors for the elemental Green strains E_{xx} , E_{yy} , and E_{xy} are 6.5, 6.9, and 5.0% strain, respectively. These relatively large RMS errors are due to the number of reasons. For one, the fitting parameters were not tuned to minimize the strain RMS errors (Nagel et al. 2014). Also, the existence of high-strain regions results in substantial errors if there is even a small difference in the location of high strain between the model and the experiment.

3.3 Model predictions

In this section, we examine a series of field variables from the model to assess the heterogeneous multiscale mechanical response of the tissue. We look at the heterogeneity in both the macroscale (tissue level) and the microscale behavior. All of the results in this section are for the strip biaxial test in L–M direction.

At the macroscale, the heterogeneous fiber structure leads to heterogeneous strain and stress fields. The local fiber orientation with respect to the global loading direction determines the relative stiffness of a given location, hence determining the amount of strain and stress generated therein. This effect leads to a heterogeneous mechanical response, which happens even with the same mechanical properties uniformly distributed throughout the sample. The field (strain/stress) heterogeneity was assessed by measuring the ratio of maximum to the mean value over the entire model domain. This ratio, named the “degree of locality,” was calculated and plotted in Fig. 6 for the maximum first principal Green strain and maximum first principal Cauchy stress fields. These values are 2.35 ± 0.30 (mean $\pm$ 95% CI) and 3.28 ± 0.24 for strain and stress, respectively. To minimize the edge effects, the boundary elements were not included in the calculations. Because of the nonlinearity in stress–strain relationship and the high level of anisotropy, the degree of locality for the maximum principal Cauchy stress is greater than that for the maximum principal Green strain. For either of these fields, the peak values are significantly higher than the average ones, emphasizing the high degree of heterogeneity. For comparison, the simulations were repeated for a structurally homogenous tissue model with fiber alignment equal to an averaged alignment (along L–M direction); for those cases, the degree of locality was

1.46 ± 0.15 (mean $\pm$ 95% CI) for maximum principal Green strain and 2.52 ± 1.13 for maximum principal Cauchy stress. These values are also shown in Fig. 6.

Also at the macroscale, Fig. 7a–c shows the local stretch ratio along the L–M direction (λ_{xx}) and averaged fiber stretch ratio ($\langle \lambda_{\text{fiber}} \rangle^e$) as probability histograms. Although one might expect the stretch ratios to be uniformly distributed for a nearly uniaxially stretched tissue (strip biaxial test in L–M), the histograms show wide ranges over the tissue's domain.

Completing the histogram, the λ_{xx} map for a representative sample is shown in Fig. 7b. Arrows on the surface of the sample show the local fiber orientation from the imaging data. Two points along approximately the same horizontal line were selected and connected to their corresponding point on the probability distribution histogram. Although these two points are on the same horizontal line, they experience very different stretches along this horizontal direction. The point on the right has fibers aligning more toward the S–I (vertical) direction, which make that region more compliant to stretch in L–M (horizontal) direction. These results were seen generally for most of the regions with high λ_{xx} , as can be seen from Fig. 7b. The variation in λ_{xx} represents an example of heterogeneity in the macroscale response which arises from structural heterogeneity.

The $\langle \lambda_{\text{fiber}} \rangle^e$ map for a representative sample, as shown in Fig. 7D, suggests the similar conclusion. Similarly, two points along approximately the same horizontal line were chosen and connected to their location on the probability distribution histograms. Averaged fiber stretch ratio changes throughout the sample in an opposite way, with regions containing fibers aligned toward the S–I direction tending to experience lower fiber stretch ratios, because of loading in L–M direction. Note that for both λ_{xx} and $\langle \lambda_{\text{fiber}} \rangle^e$, regions closer to the superior and inferior boundaries experience lower stretches because they are under the effect of these fixed sides.

The heterogeneity was also studied at the microscale (RVE level). For this purpose, two elements were selected (Fig. 7f), and the probability distribution of stretch ratios for all the fibers inside each element is shown in Fig. 7e. Each histogram shows a wide span of fiber stretch ratios, which suggests the high degree of heterogeneity on the microscale. In other words, the heterogeneous structure leads to a non-uniform response in the tissue level, and the microstructure at each location itself undergoes a non-uniform deformation.

4 Discussion

In this study, we performed entire-domain fiber imaging of flattened dissected lumbar FCLs ($n = 6$) and used the imaging data to construct image-based multiscale models to investigate the macro- and microscale mechanical behavior of lumbar FCL during in vitro deformations. The models were developed based on local fiber structure measured *vis a PS-OCT*. For each lumbar FCL sample, the computational results were then compared to three tests (equibiaxial, strip biaxial in L–M direction, and strip biaxial in S–I direction) performed on each sample. In terms of gross force–strain behavior, our model showed a relatively good fit to three separate experimental results, by using only one set of three fitting parameters for each sample. In terms of local behavior, the model showed the ability to capture high-strain

regions from the experiments qualitatively. These results demonstrate the importance of incorporating heterogeneity into a model if one intends to predict the local response of the FCL to an applied load. This model predicts that the heterogeneous structure gives rise to a non-uniform response at both macro- and microscale, as shown in different panels of Fig. 7. Macroscopic (maximum principal Cauchy stress and Green strain, tissue stretch ratio, and averaged fiber stretch ratio) and microscopic (fiber stretch ratio) strain-based measurements reveal the considerable degree of heterogeneous response at both scales. Our results suggest that a detailed fiber structure governs the heterogeneous mechanical response of lumbar FCL at multiple scales, and therefore, a microstructurally informed model must be used to capture the local behavior of lumbar FCL during in vitro tests or more complex loadings in vivo, which is the case for the lumbar FCL's physiological deformation (Ianuzzi et al. 2004; Little et al. 2004; Ianuzzi and Khalsa 2005; Claeson and Barocas 2016).

We observed a heterogeneous fiber structure over the lumbar FCL. In a previous study, Yamashita et al. (1996) used light microscopy; based on qualitative observations, they reported that fibers run in the L–M direction in the superior and middle parts of the lumbar FCL, and in the superior–medial to inferior–lateral direction in the inferior regions. Our imaging results confirm the L–M direction as the primary fiber orientation, and the calculated average fiber orientation values for each region are in agreement with what Yamashita et al. qualitatively described. Our results, however, significantly expand Yamashita's findings by quantifying the detailed entire-domain fiber structure of each specific sample. These results are also in agreement with those of our group's previous (Claeson et al. 2015) observation of a heterogeneous structure in the lumbar FCL based on OCT imaging. The nonuniformity of fiber organization in lumbar FCL, as well as the resulting stress and strain localization, could predispose certain regions of the tissue to injury and thus have significant clinical impact.

Low back pain (Cohen 2007) can be considered in terms of mechanical stimulus of tissue constituents (e.g., mechanoreceptors), a process that is itself dependent on the local mechanical environment within the lumbar FCL. The lumbar FCL has a complex 3D geometry and undergoes complex deformations under spine motion, and those deformations in turn lead to heterogeneous stresses and strains within the tissue. Our image-based model predicts a highly non-uniform response at two scales, a result that highlights the significance of tissue's structural heterogeneity. The next, more challenging, step in understanding lumbar facet capsule pain is connecting the heterogeneous multiscale deformation field to the deformation and possible injury of neurons embedded within the tissue.

Before concluding the discussion, we address briefly a number of limitations to this study. Perhaps the most significant limitation is the relatively low depth of imaging. The lumbar FCL is a thick tissue, with the average thickness of 2.5 mm (Claeson and Barocas 2017). Optical penetration depth, however, allowed us to capture its structure for only the first 0.13 mm from the surface. Nevertheless, this shallow depth is greater than that obtainable by SEM and overcomes the challenges of a transmission-based approach. An additional simplification was the assumption that fibers run nearly parallel to the sample's surface. The *en-face* fiber orientation field, which was a 2D projection of the actual 3D fiber orientation, was used without correction for three dimensionality. This problem could be overcome by

slicing the sample followed by a 3D reconstruction of z-stacks, as previously performed on aortic tissues by Schriebl et al. (2013), or by using computational (Wang et al. 2015) or experimental methods (Ugryumova et al. 2006; Liu et al. 2016) from PS-OCT contrasts.

In terms of gross force–displacement behavior, our imaged-based model resulted in unsatisfactory agreement for two of the samples. We attribute this to lack of dominant fiber orientation in the L–M direction (as was observed in the other samples) and to the low number of fitting parameters used for three mechanical tests of each sample. Therefore, the fitted parameters obtained could be limited by the relatively low sample size. Two major strengths of this modeling approach are its small number of fitting parameters and direct inclusion of structural data. These strengths, however, are not without a considerable cost; because the model relies on structural data and has no structural fitting parameters, inconsistency between structural imaging and mechanical behavior cannot be resolved. This problem, which is exacerbated by limited imaging capacity (e.g., the relatively small imaging depth for our PS-OCT system), led to poor fitting for two of our six samples.

Assuming the same fiber and matrix properties throughout each sample was another important simplification of this model. As the SEM images of Fig. 1 suggest, fibers have a range of diameters, and consequently a range of mechanical properties and densities at different locations on the lumbar FCL. In addition, the ventral layer of the lumbar FCL contains isotropic elastin fibers (Yamashita et al. 1996), which are more compliant compared to collagen fibers. In our models, however, we assumed the same fiber properties (stiffness, density, etc.) throughout the sample, where fibers were assumed to have the alignment of the dorsal collagenous layer because of limited imaging depth. We would expect that the elastin-rich layer, being isotropic and highly elastic, would incorporate into the neo-Hookean non-fibrillar component during fitting of the model in experimental data. The density and stiffness of other non-fibrillar tissue components could also vary depending on the location, but they were modeled as uniform lest we introduce an undue number of potential fitting parameters. Tissue viscoelasticity, which is known to play a role at some time scales (Little and Khalsa 2005; Troyer and Puttlitz 2012), also was not considered in the current model. Thus, although the current work demonstrates the importance of measuring and using structural heterogeneity when one attempts to simulate tissue behavior, the model presented here is by no means complete.

We also note that the number and/or spacing of the RVEs are important concerns. Our modeling approach assumes smooth variations among the RVEs, which means that the RVE size and spacing must be smaller in length scale than variations in structure. In the current study, the RVE length scale was 10–30 μm , and the RVE spacing (i.e., the distance from one RVE to the nearest other RVE) was roughly 500 μm . These distances are, respectively, lower than and comparable to the computed structural correlation lengths for lumbar FCL (~ 560 μm), as reported by Ban et al. (2017). Structural correlation length is the measure of the size of subdomains over which the fiber orientation field is close to being constant. So we consider the RVE resolution satisfactory. Since the RVEs are at the Gauss points of the model, an additional question—general to FEM and not specific to the multiscale approach—is whether macroscopic-scale gradients are captured adequately by the mesh scale. The

success of the mesh convergence study described earlier in the paper demonstrates that the mesh is sufficiently resolved.

In conclusion, we observe that the lumbar FCL is a complex tissue with heterogeneous organization at multiple scales. One specific aspect of that organization—the alignment of collagen fibers within the superficial dorsal layer of the tissue—can be observed optically via PS-OCT and can serve as the basis for an image-based model of the lumbar FCL. We found that the high level of detail obtainable in this manner was necessary to describe local variations in the mechanical environment, which could be critical in understanding the mechanobiology of the lumbar FCL. Such details can only be captured by a heterogeneous, multiscale model such as the one used herein. This model emphasizes the significance of multiscale heterogeneity in two ways: First, heterogeneity results in the existence of regions with stresses/strains considerably higher than those being applied on average on the tissue's boundaries, which could be significant in terms of injury. Second, the local response extracted from such a model can be further employed to explore behavior of microstructural components (e.g., neurons), a next step in the study of low back pain.

Acknowledgments

This work was supported by the National Institutes of Health (U01EB016638). We acknowledge the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources used for our computational methods. SEM images were taken at the Characterization Facility at the University of Minnesota, which receives partial support from NSF (MRSEC program).

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Appendix 1: Example of the eigenvectors of the orientation tensor

Figure 8 gives two examples to show how retardance (degree of anisotropy) can be related to the difference between eigenvalues of the orientation tensor. The figure shows two SEM images representing the fiber structure in two regions of a lumbar FCL sample. Fibers are strongly aligned in Fig. 8a, but Fig. 8b shows a nearly isotropic fiber structure. A fast Fourier transform (FFT) approach (Sander and Barocas 2009) was used for each image to calculate the orientation tensor. Yellow arrows show the eigenvectors of the orientation tensor, such that the length of each vector is proportional to its corresponding eigenvalue. The stronger alignment of fibers (higher retardance) corresponds to larger difference between eigenvalues.

Appendix 2: Additional details of the microscale finite element method

A brief description of the method is provided here. For full details, refer to (Chandran and Barocas 2007; Stylianopoulos and Barocas 2007). In this two-scale finite element method, the final macroscopic equilibrium equation is derived based on microscopic balance and is expressed as:

$$S_{ij,i}^{\text{fib}} + S_{ij,i}^{\text{mat}} = \frac{1}{V} \oint_{\partial V} (s_{ij}^{\text{fib}} - S_{ij}^{\text{fib}}) u_{k,i} n_k dS \quad (8)$$

where S^{fib} , s^{fib} , and S^{mat} are the (macroscopic) averaged Cauchy stress tensor of the fibers, the microscopic the Cauchy stress tensor of the fibers, and the macroscopic stress tensor of the non-fibrillar matrix, respectively. The stress due to the non-fibrillar matrix was assumed to vary only on the macroscale. Also, u_k , n_k , and V are the displacement of the RVE boundaries, the surface normal vectors for the RVE, and the RVE volume, respectively. The integral is calculated over the boundary surfaces of the RVE. The additional term on the right-hand side is due to non-uniform stress-deformation of the RVE boundaries.

The average Cauchy stress tensor of the fibers can be obtained based on network fiber forces, according to the volume averaging theory and under the assumption that fibers are of negligible cross-sectional area:

$$S_{ij}^{\text{fib}} = \beta \cdot \frac{1}{V} \sum_{\substack{\text{boundary} \\ \text{fiber nodes}}} x_i f_j$$

where x is the position of the fiber nodes located on the RVE boundaries and f is the force of the corresponding fiber. β is a stress conversion factor between the micro- and macroscale, and was calculated for RVEs containing ~400 fibers occupying ~10% of their volume.

Appendix 3: Example of tensor-based averaging

Figure 9a, b compares the fiber orientation distribution on the fine field (OCT results) and on the coarse finite element grid after tensor-based averaging via Eq. (6) for one sample. The fiber orientation distribution over the entire domain is visually similar. A plot of the fiber distributions (Fig. 9c) confirms the agreement between the fine and coarse fields.

Fig. 1.

SEM images at different locations over the lumbar FCL surface show a high degree of heterogeneity of fiber organization. Fibers also have various diameters in this tissue. All images were taken at the same sample orientation, with superior–inferior directions being vertical and lateral–medial direction being horizontal. The tiles of SEM images do not represent the actual location indicated by *letters* in the *upper image*

Fig. 2.

Schematic of two scales and their connection in the multiscale method. Each finite element (in macroscale) consists of eight Gauss integration points, and on each Gauss point, an RVE (in microscale) is located. RVEs comprise a fiber network along with a neo-Hookean matrix which deforms independently of the fiber network

Fig. 3.

a Normalized probability distribution of fiber orientation for six samples. The reference angle is the L–M direction. Although the peak orientation is the L–M direction for most of the samples, fibers are oriented in a wide range of orientation angles over each sample. **b** Fiber orientation fields for two representative samples. The direction of each vector shows the fiber orientation, and the length of each vector is proportional to the local strength of alignment. Both fields show a considerable level of heterogeneity. In some regions, fibers are aligned and in some regions there is a more isotropic distribution of fibers

Fig. 4. Grip force versus grip strain results of the experiment ($n = 6$) and the model for the **a** equibiaxial, **b** strip biaxial in L–M direction, and **c** strip biaxial in S–I direction tests. Symbols show average experimental results (error-bars are 95% CI), and solid lines show average model results. Red and blue curves show forces in L–M and S–I directions, respectively. In average, the model predicts the experiment with good accuracy in all directions. **d** Best-fit parameters for the model (error-bars are 95% CI). Fiber nonlinearity parameter (**b**) had slight variations among different samples, while the fiber stiffness parameter and shear modulus of the Neo-Hookean matrix changed over a wider range

Fig. 5. Green strain E_{xx} and E_{yy} for the experiment and the model for two representative samples during equibiaxial extension. In general, the heterogeneous model predicts the approximate location of high-strain regions; however, the accuracy of this prediction is different over different samples

Fig. 6.

Degree of locality, measured as the ratio of maximum to mean value, calculated for the maximum principal Cauchy stress and maximum principal Green strain, for two models: the model with heterogeneous fiber alignment based on imaging data and the model with homogeneous fiber alignment equal to the average measured alignment. The *columns* show the average value for all sample ($n = 6$), and the *error-bars* are 95% CI. The degree of locality is relatively high for both of these fields. Cauchy stress is more localized compared to the Green strain. The heterogeneous model results in greater degree of locality for both Green strain and Cauchy stress

Fig. 7.

This figure represent macroscopic (**a–d**) and microscopic (**e** and **f**) heterogeneity for lumbar FCL samples during strip biaxial test in L–M direction. *Blue* vectors on the surface show the local fiber orientation. *Black* vectors on the *left* and *right* boundaries show the boundary displacements. **a** Probability distribution of stretched along the L–M direction (λ_{xx}) for $n = 6$ samples (*solid line* for representative sample curve). **b** Map of λ_{xx} for a representative sample. Although samples are pulled nearly uniaxially, a wide range of stretches exists over the domain. For a representative sample, two selected point at around the same vertical level show the variability in stretches. Regions with fibers aligned toward the vertical direction show higher compliancy to stretch. **c** Probability distribution of elemental averaged fiber stretch ratio ($\langle \lambda_{\text{fiber}} \rangle^e$) for $n = 6$ sample (*solid line* for representative sample curve). **d** Map of $\langle \lambda_{\text{fiber}} \rangle^e$ for a representative sample. Similarly, two elements are selected to show the heterogeneity in fiber stretches. Unlike λ_{xx} , regions with more vertical orientation experience higher fiber stretches. **e** Probability distribution of stretch ratios (λ_{fiber}) for fibers inside two selected elements. **f** Two selected elements to represent the fiber stretch ratio's

variability. The histograms for these two elements show the microscale heterogeneity, where even fibers within an element experience a relatively wide range of stretches

Fig. 8.

Two SEM images of collagen fibers on a lumbar FCL sample shows two different fiber organization, **a** highly aligned fibers, and **b** an isotropic fiber distribution. The orientation tensor for each image was calculated using fast Fourier transform (FFT). The *yellow* vectors show the eigenvectors of the orientation tensor and the length of each vector is proportional to their corresponding eigenvalues. The higher difference between the eigenvalues represents the higher degree of anisotropy

Fig. 9.

a Fine-resolution fiber orientation field based on PS-OCT measurement for one representative sample. **b** Fiber orientation field over a coarse finite element grid over the same sample as a result of proposed tensor-based averaging. Fiber orientation distributions are visually similar on all location over the two fields. **c** Fiber orientation histograms for the fine and coarse grids. These histograms verify the great accuracy of the tensor-based averaging method