Collagen Organization in Facet Capsular Ligaments Varies With Spinal Region and With Ligament Deformation

The spinal facet capsular ligament (FCL) is primarily comprised of heterogeneous arrangements of collagen fibers. This complex fibrous structure and its evolution under loading play a critical role in determining the mechanical behavior of the FCL. A lack of analytical tools to characterize the spatial anisotropy and heterogeneity of the FCL's microstructure has limited the current understanding of its structure–function relationships. Here, the collagen organization was characterized using spatial correlation analysis of the FCL's optically obtained fiber orientation field. FCLs from the cervical and lumbar spinal regions were characterized in terms of their structure, as was the reorganization of collagen in stretched cervical FCLs. Higher degrees of intra- and intersample heterogeneity were found in cervical FCLs than in lumbar specimens. In the cervical FCLs, heterogeneity was manifested in the form of curvy patterns formed by collections of collagen fibers or fiber bundles. Tensile stretch, a common injury mechanism for the cervical FCLs, heterogeneity was manifested in the form of curvy patterns formed by collections of collagen fibers or fiber bundles. Tensile stretch, a common injury mechanism for the FCLs, significantly increased the spatial correlation length in the stretch direction, indicating an elongation of the observed structural features. Finally, an affine estimation for the change of correlation length under loading was performed which gave predictions very similar to the actual values. These findings provide structural insights for multiscale mechanical analyses of the FCLs from various spinal regions and also suggest methods for quantitative characterization of complex tissue patterns.

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1 Introduction

The spinal facet joints are complex intervertebral anatomical structures whose injury can lead to pain [1–6]. The facet joints are positioned bilaterally and symmetrically on the posterolateral aspects of each spinal motion segment and provide the articulation of the lateral mass of adjacent vertebrae (Fig. 1). Like the other synovial joints, the spinal facet joint is fully enclosed by a capsular ligament. The FCL provides mechanical support, and couples and resists motions to help to guide the relative movement between the articular facets. The FCL is mainly composed of dense collagen fiber bundles and elastin fibers that withstand the tensile and shear loading [1,6,7], such as that which occurs during the spinal loading and motion [8–10]. Differences in the loading demands for the cervical and lumbar FCLs are attributable to many factors: varied spinal motions, distinctive ligament geometry, and distinctive vertebral anatomy in different regions across the spine [1,9,11]. For example, the orientation angles of the cervical facet articulation are 70–96 deg with respect to the sagittal plane and 20–78 deg with respect to the axial plane [12,13]. In contrast, the lumbar facets are oriented 15–70 deg relative to the sagittal plane and 82–86 deg relative to the axial plane [1,14]. In addition to their macroscopic differences, the FCLs in the cervical and lumbar regions may have dissimilar microscopic architectures that...
Fiber orientation has been suggested by numerous previous ex vivo collagen fibers constitute the major component of the FCL, collagen microstructure, which has enabled studies to define the relationships between microstructure and mechanical functions [15–19]. Since polarized-light-based tools, it has become possible to study the FCL microstructure, enabling studies to define the relationships between microstructure and mechanical functions [15–19]. Since collagen fibers constitute the major component of the FCL, collagen fiber orientation has been suggested by numerous previous ex vivo and in vitro studies to play a key role in FCL mechanics [6,16,19]. Although there are histological studies showing that both the cervical and lumbar FCLs contain regions with parallel and unaligned fibers [4,6], evidence of the anisotropy and heterogeneity in collagen organization for an entire ligament and across FCLs from different spinal levels is very limited. This information is critical in understanding, and possibly explaining, the mechanical behavior of the FCL, especially the nonuniform capsule strains of the human cervical and lumbar facets under flexion, extension, bending, and rotation [1,8,11,20].

Beyond its mechanical function, the sensory function of the FCL may also be affected by its fibrous microstructure. The FCL has been increasingly recognized as a sensory organ due to its innervation by mechanoreceptors and nociceptors [2,4,21,22]. Indeed, it has been identified as a common source of posttraumatic neck pain and chronic lower back pain [1,2,23]. Nerve fibers that innervate regions of the FCL with irregular or highly organized collagen fibers may be injured to different extents when the FCL undergoes large, heterogeneous deformations. The previous ex vivo and in vitro studies have provided evidence for the dependence of nociceptive signaling on the orientation of collagen fibers surrounding the neurons [17,24]. Specifically, local collagen disorganization has been observed during loading of the cervical FCLs at strains that induce pain in vivo [16,25,26], and stretch-induced collagen realignment affects the nociceptor activation in vitro neuron–collagen constructs [24]. To begin to understand the relationships between collagen fiber kinematics and possible neurophysiological effects, and to identify injury-prone regions in the FCL for potential treatment and/or protection, the fibrous structure of the FCL and its response to loading must be characterized. Thus, two hypotheses were evaluated in this work: (1) that the tissue-level heterogeneity of the collagen fiber network in the FCL varies between the cervical and lumbar regions, and (2) that structural differences become more pronounced when the tissue is stretched.

2 Materials and Methods

2.1 Quantitative Polarized Light Imaging of Cervical Facet Capsular Ligaments. The fiber orientation in cervical FCLs at rest in a prestressed unstretched configuration and during loading were obtained previously using a QPLI system [15,16]. This system was integrated with a tensile testing machine (Instron, Norwood, MA) and acquired real-time pixelwise collagen alignment maps during loading, based on the linear birefringence of collagen fibers [16,27]. Briefly, FCL specimens from the C4/C5 joints (n = 7; age 63 ± 15 yr; two females) were prestressed to 5 kPa (Fig. 1(b)). Polarized light images were acquired at 12.5 pixel/mm resolution in the preloaded reference position and during continuous tensile loading until ligament failure occurred. The measured light intensity was fit to a harmonic equation; the average fiber orientation direction and the strength of the fiber alignment in the mean alignment direction (measured as the retardation) at each pixel were determined based on the phase and amplitude of the harmonic responses [25,27]. Fiber alignment maps (Fig. 1(c)) were created at increments of 1 mm displacement (approximately 1.18 stretch ratio) until ligament failure at approximately 1.55 stretch ratio; detailed mechanical information for each specimen is provided in Table 1. Spatial maps of collagen fiber orientation before and during tensile loading were analyzed via autocorrelation analysis as described below.

2.2 Optical Coherence Tomography of Lumbar Facet Capsular Ligaments. Because the lumbar FCL is roughly five times thicker than the cervical FCL (Table 1) and less transparent,
QPLI is not a viable imaging tool for measuring the collagen matrix organization in the lumbar FCL. However, the polarization-sensitive optical coherence tomography (PS-OCT) can be effective for structural characterization of the lumbar FCL [28]. As such, PS-OCT was used to image lumbar FCL samples in a prestressed resting state [19]. Briefly, lumbar L4/L5 FCL specimens (n = 6; 51 ± 12 yr; one female) were clamped biaxially and pulled with ~1 N force on each side to make them as flat as possible (Fig. 1(a)). Samples were then imaged using the PS-OCT system (resolution of ~250 pixel/mm), with multiple images being tiled together to produce a single image of fiber orientation over the entire lumbar FCL sample (Fig. 1(c)). The phase retardance and optic axis quantified the strength of fiber orientation over the entire lumbar FCL sample (Fig. 1(c)).

The average thickness for cervical FCLs is 0.42 ± 0.07 mm; the average thickness for lumbar samples is 2.65 ± 0.30 mm [37].

### Table 1 Sample information of cervical and lumbar FCLs

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<th>Rupture displacement (mm)</th>
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*a The average thickness for cervical FCLs is 0.42 ± 0.07 mm; the average thickness for lumbar samples is 2.65 ± 0.30 mm [37].

*b Width and length correspond to the medial–lateral and superior–inferior directions, respectively.

### 2.3 Spatial Autocorrelation Calculation

The orientation imaging provides the fiber direction (x) at each point located at x in the two-dimensional region being imaged. For simplicity, we used cosine of the orientation angles to study the correlation in the orientation fields. The autocorrelation [29] of the orientation angle’s cosine at a distance δ can be defined as follows (Fig. 2):

$$R(\delta) = \frac{E[c'(x)c'(x+\delta n(\theta))]}{\sigma^2}$$

where E is the expected value operator, which averages over all values of θ and vectors x in the domain. The vector n is of unit length and points in the direction given by θ, that is, the vector [cos θ, sin θ]. So, $E[c'(x)c'(x+\delta n(\theta))]$ is the average of the product of c' values for all possible pairs of pixels located at a distance δ. The function c' measures the deviation of an orientation from the average value, i.e., $c'(x) = \cos(x(x)) - E[\cos(x(x))]$. The expression $E[\cos(x(x))]$ gives the average of the cosine of all orientation values in an orientation map, so $E[c'(x)] = 0$, and $R(\delta)$ is greater than zero if the fiber orientation angles at points a distance δ apart are positively correlated. R is normalized by $\sigma^2$, the variance of $\cos(x(x))$ over the problem domain, so $R = 1$ for perfectly correlated orientations.

The autocorrelation function defined in Eq. (1) includes averaging over all possible directions of the unit vector n, i.e., over $\theta \in (0, \pi)$. To numerically calculate $R(\delta)$, the expected value, $E$, was evaluated by starting from a point inside the sample, located at x, and calculating $c'(x)c'(x+\delta n(\theta))$ for every pixel located at a distance δ. Then, x was changed to a new pixel, and the calculation was repeated (Fig. 2(b)). After trying all possible pixels for x, the expected value was found by averaging all evaluated values $c'(x)c'(x+\delta n(\theta))$.

In some cases, one is particularly interested in a specific direction, such as the bone-to-tendon direction or the loading direction. In this case, the autocorrelation measure for a specific direction $\theta$, $R(\delta, \theta)$, becomes

$$R(\delta, \theta) = \frac{E[c'(x)c'(x+\delta n(\theta))]}{\sigma^2}$$

In Eq. (2), the expected value was calculated by starting from a point at x, finding another point at distance δ and orientation θ,
tracking the position of the fiduciary markers (Fig. 1), the principal strain across each four-node elements was computed by dividing the sample’s relative change of area by the vertical stretch ratio. The affine estimation was evaluated by resizing the height and width of each pixel by the FCL vertical and horizontal stretch ratios, respectively, while accounting for the rotation of the pixel’s orientation. For example, if a hypothetical square pixel had dimensions 0.1 mm and an orientation value of π/4 rad before deformation, and if the sample was stretched to twice its size in the vertical direction without contracting in the lateral direction, the pixel would deform into a rectangle 0.1 mm in width and 0.2 mm in height. The pixel’s orientation would be transformed affinely to 1.11 rad. We repeated this procedure for all pixels to calculate an affinely transformed orientation map. We evaluated the autocorrelation functions using these maps and calculated affine estimations of the correlation lengths.

3 Results

3.1 Anatomic Regional Dependence of Collagen Architecture. FCLs from the cervical and lumbar spines exhibit different collagen fiber organization; high intra- and intersample variability was observed in the cervical FCLs (Fig. 3). Each cervical FCL contains regions with heterogeneous fiber orientation and areas comprising parallel collagen bundles with strong alignment as inferred from QPLI-based orientation maps (Fig. 3). The strongly aligned collagen fiber collections have different orientations in different regions of the FCL, and some of them together form curvy patterns (Figs. 3(b)–3(d)). In contrast, although local heterogeneity in collagen orientation is also observed in lumbar samples, the majority of the fibers in lumbar FCLs align in the across-joint direction, with alignment strength more uniform than that observed in cervical samples (Fig. 4). Among the six lumbar samples examined, five have probability density functions of the PS-OCT preferred angle peaking near 0 deg, which corresponds to the medial–lateral direction (Fig. 4(a)). In contrast, the cervical FCLs show no consistency in their collagen orientation probability density functions (Fig. 3(a)).

Differences in collagen architecture between cervical and lumbar FCLs are captured quantitatively by the computed spatial
The tensile loading results in minor perturbations of the horizontal autocorrelation in individual ligaments (Fig. 6(b)) and to a slight increase of the autocorrelation range in the vertical, loading direction. This indicates that collagen reorientation takes place across the sample, although with different intensities in various subdomains. The initial heterogeneity of the cervical ligament orientation persists during loading, which is demonstrated by the clear strain invariance of the \( \theta \)-averaged autocorrelation (labeled isotropic in Fig. 6(b)).

These trends can be described more quantitatively using the correlation length which provides a measure of the size of the subdomain over which collagen orientation is approximately identical. For the four cervical ligaments that were not damaged before 3 mm of the stretch (Table 1), the vertical feature size measured at 3 mm displacement is significantly larger (\( p < 0.050 \)) than that of the prestressed unstretched configuration and greater (\( p < 0.006 \)) than the horizontal feature size at 3 mm stretch (Fig. 7(a)). The correlation length in the horizontal direction slightly decreases with increasing deformation, but this change is not statistically significant (Fig. 7(a)). Decreases in the horizontal feature size with increasing strain are only observed in four of the seven samples (Fig. 7(b)). In contrast, all samples exhibit a trend of increasing vertical feature size with increasing maximum principal strain (Fig. 7(c)).

3.3 Nonaffinity in Ligament Deformation. In a uniaxial stretch test, fibrous networks deform in a highly nonaffine manner [32,33], marked by deviations from the externally applied strain at each point in the sample. This motivated a comparison between the measured correlation lengths against values calculated based on hypothetical affinely deformed samples. Like the actual correlation lengths, the affinely calculated correlation lengths increase...
in the loading direction. However, they do not follow the small decreasing trend observed in the correlation length in the lateral direction. Overall, the affinely deformed maps result in correlation lengths that are close to the actual values (Fig. 8).

### Discussion

Tissue microstructure, such as collagen organization in ligaments, plays an important role in mediating mechanics and physiology of the tissue [16,17,19,24,25,34–36]. In this study, we utilized analytical tools common in the analysis of random functions to investigate tissue patterns, collagen architecture in particular, in the facet capsular ligament. The FCL plays an important role in the biomechanics of the facet joint and is a common source
of neck and lower back pain from excessive capsule stretch [1,2]. Uniaxial tension at supraphysiologic strains can induce abnormal collagen reorganization in subregions of the cervical FCL and concurrent activation of nociceptors embedded in the impaired fibrous networks [2,16,17,26]. Equibiaxial loading of lumbar FCL has shown to produce normal and shear forces [37]. The lumbar FCL undergoes extension and shear due to physiological loadings such as spinal torsion (primarily extension), lateral bending (primarily shear), and flexion-extension (primarily shear). To capture the spatial heterogeneity of the fibrous structure in the FCL, collagen fiber alignment maps were generated using specialized optical imaging techniques, namely, QPLI and PS-OCT for cervical and lumbar FCLs, respectively. The spatial correlation analysis was performed to compare collagen organization in FCLs in cervical and lumbar regions quantitatively and to evaluate collagen reorganization during tensile loading of cervical FCLs.

Each of the different spinal FCLs displays distinct collagen organization patterns based on their anatomic region. High spatial heterogeneity and curvy substructures are observed in cervical FCLs (Fig. 3). In contrast, collagen orientation in lumbar FCLs is more homogeneous with the majority of the fibers aligning along the direction across the joint (Fig. 4). This structural difference likely associates with the different mechanical properties of FCLs in these two spinal regions. For example, the maximum principal strains, including those produced by bending, flexion, and extension, are generally larger in the cervical than in the lumbar FCL [3,38,39]. The mean failure strains during tensile loading of FCLs in the cervical spine range from 100% to 150% [3,16,40]; whereas for the lumbar FCL, the extensibility to failure is 0.65 and 0.60 in the direction parallel and perpendicular to the collagen fiber orientation, respectively [20].

Cervical FCLs can accommodate larger macroscopic deformation maybe due to differently oriented collagen fiber or fiber bundles, as suggested by the curvy substructure observed in orientation maps derived from the QPLI data (Fig. 3). Collagen fibers or fiber bundles with spatial orientations different from the loading direction can first rotate toward the loading direction before getting stretched, a process analogous to the straightening of a single wavy fiber. Of note, the current study did not measure the waviness of the collagen fibers, because the orientation obtained here is the optical anisotropy viewed by polarized light, which indicates, but is not the same as, the collagen fiber direction, and the images suggest the average fiber alignment at each pixel rather than fiber-level orientations.

On the other hand, the predominant lateral–medial fiber orientation in the lumbar FCL provides it with the mechanical strength and stiffness necessary to bear the large extension and shear loads required for daily activity. During torsion or lateral bending, the lumbar FCL experiences extension along the joint direction (lateral–medial), and thus, the lateral–medial fiber orientation stiffens their structure in a functionally relevant direction. During extension or flexion, the lumbar FCL also undergoes substantial shear, transverse to joint direction [37,38,41,42]. Fiber alignment in the joint direction also provides resistance in tissue shear since fibers resist more when sheared transverse to their direction, as compared to when sheared along their axis of orientation.

It has been shown that fiber orientation in the lumbar FCL affects the tissue’s stress–strain behavior in tension, and different mathematical models have been used to describe facet capsules loaded in directions parallel and perpendicular to the collagen fibers [19,20]. Similar to the lumbar region, inhomogeneous collagen organization in the cervical FCL also likely results in varied mechanical behaviors under loading in different directions and requires future mathematical descriptions that can account for the spatial variability in collagen organization. Although this study demonstrates spinal region-dependent microstructure that potentially relates to FCL mechanical function, it remains unclear whether these structural characteristics are inherent or result from adaptation during maturation and aging. Thus, the causal relation ships of the structure–function coupling need to be further elucidated.

The fiber orientation autocorrelation along different directions of the cervical and lumbar FCLs both exhibit rapid decay within 1 mm, with a mean isotropic (average direction) correlation length of 0.35 mm for cervical FCL and 0.56 mm for lumbar FCL, which corresponds to approximately 5% and 3% of the size of the cervical and lumbar samples, respectively (Fig. 5; Table 1). The correlation length for the cervical FCL drop is exponential with distance, but the lumbar correlation drops by about 70% within 1 mm and remains relatively stable with a long tail above 0 between 1 mm and 3 mm (Fig. 5). This lack of spatial correlation of inferred collagen orientation in the cervical FCL on the scale larger than the correlation length suggests lack of periodicity in the observed microstructural patterns, more randomness, and higher structural isotropy at the ligament scale. These findings are consistent with the spatial variation of collagen alignment observed in the cervical FCLs (Figs. 3 and 4).

In addition to intrasample heterogeneity, cervical FCLs also exhibit high interspecimen variability. No two cervical samples show identical fiber orientation distributions (Fig. 3). In contrast, five of the six lumbar samples display an orientation distribution with a peak in the joint direction (Fig. 4). These results suggest that sample-specific computational models may be needed to correctly represent the structure of cervical FCLs. Since collagen fiber alignment may affect the mechanical behavior of tissues [16,19,35,36], it is important to incorporate fiber orientation into computational models of the FCLs, especially cervical FCLs.

Spatial alignment maps derived from polarized light images of the cervical FCL during loading suggest complex collagen reorganization patterns, with collagen within some subdomains realigning toward the loading direction, while that in other subdomains rotating in the direction perpendicular to the direction of average maximum principal stretch (Fig. 6). In addition to heterogeneous reorganization within each individual sample, different FCLs show different realignment responses. These observed behaviors are likely due to varied initial orientation and slightly different FCL geometries, and they may contribute to the inhomogeneous strain field and different failure locations observed during tensile loading of FCL specimens [16,25]. Besides the shape and structure of the FCL itself, surrounding muscles may also contribute to the heterogeneity of this ligament. For example, the cervical facet capsule has nonuniformly distributed muscle insertions that cover approximately 23% of the capsule [43], which also locally alter the stress and strain responses in the different and overall regions of that capsule and also across different specimens. Further characterizing these and other physiological and anatomic inputs to the FCL would go a long way in better understanding the mechanical function of this ligament.

Structural reconfiguration of collagen during loading of the cervical FCL was quantified by extracting a correlation length, representing a characteristic feature size, from the autocorrelation data. At large (vertical) displacement, the cervical FCLs show significantly greater correlation lengths in the vertical direction than in the horizontal direction and in the prestressed resting configuration (Fig. 7(a)). This finding is consistent with multiple previous observations that tensile loading realigns collagen fibers in soft tissues in the principal strain direction [27,36]. The mean vertical feature size exhibits a 75% increase, while the samples undergo an average 55% stretch (i.e., 1.55 stretch ratio; Fig. 7(a) and Table 1), suggesting that the substructure size changes more rapidly than the imposed deformation of the ligament. All seven samples have increased vertical feature size as the applied strain increases (Fig. 7(b)); however, changes in the horizontal correlation length are unexpectedly variable (Fig. 7(c)). The small change in feature size in the horizontal direction is accompanied by small lateral contractions observed in these experiments. This may be explained by the bone constraining the induced deformation and minimizing lateral contraction during extension of these samples (apparent Poisson’s ratio ≈ 0.08 ± 0.26).

The affine model predicts vertical correlation lengths that are in very good agreement with our experimental measurements, indicating substantial homogenous stretches in this direction.
cal microscopy and scanning electron microscopy \[30,44\], and it other imaging techniques at different length scales, such as confocal visualization, reveals the dependence of the collagen organization. First, the interpretation of the results of any image-based analysis can be applied to the complex fibrous tissue or tissue analogs at rest and in response to loading. This work was supported by funding from the NIH (Grant No. U01-EB016638) and a Force and Motion Foundation Scholarship (S.Z.). The authors thank Dr. Kyle Quinn for performing the experiments on cervical FCLs that provided the QPLI data used for the spatial correlation analyses.

References


