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Application of Quantitative Polarized Light Techniques for Characterizing Ligament Fiber Kinematics during Facet Joint Loading

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ABSTRACT

While studies have reported the failure properties of the facet capsular ligament, and pointed to its potential for subfailure injury that may cause pain, the mechanical conditions and mechanisms that lead to the initiation of ligament injury remain undefined. As has been previously demonstrated for other soft tissues and ligaments, understanding the ligament's response to mechanical loading requires insight into its microstructural behavior. Therefore, this study describes the development and implementation of quantitative polarized light imaging (QPLI) to define the collagen fiber direction and strength of alignment in the facet capsular ligament. Fiber alignment maps were created during tensile loading of human cervical (C6/C7) ligament specimens and compared with simultaneous mechanical measurements. The mean fiber direction and the variance of this direction were calculated from all pixels in each image map for each time point. Retardation was also measured at each pixel to determine the strength of alignment through the thickness of the ligament. Gross ligament (n=3) failure occurred at 66.3±11.1 N and 5.84±1.81 mm. Changes in the mean fiber direction, variance, and retardation were observed prior to gross failure and minor rupture. Mean variance of fiber direction and retardation both decreased for all specimens up to gross failure. Error in measuring direction and retardation in our QPLI system was quantified as 2.93±1.45˚ and 0.85±0.44˚, respectively. These findings from pilot studies demonstrate use of integrative methods to examine the relationship between microstructural and mechanical responses of the cervical facet capsule under load. Preliminary results suggest that QPLI techniques may be useful in broadening the experimental approaches used to study this ligament and its role in injury. Further, data obtained from these types of studies could provide additional perspectives for defining more appropriate thresholds for painful injury and for constructing more biofidelic models of the facet joint.

INTRODUCTION

Chronic neck pain is a tremendous problem in the United States, affecting at least 15.5 million Americans annually, with symptoms that can lead to disability and represent nearly $30 billion in health-related expenses (Freeman et al., 1999). Whiplash injury in particular is responsible for nearly half of patient-care costs associated with injuries in motor vehicle crashes (Quinlan et al., 2004). Despite the high incidence of whiplash-associated neck pain, little remains known about the injuries producing these syndromes and the physiologic mechanisms responsible for their persistence.

The cervical facet joint has been strongly implicated as playing a role in whiplash and neck pain. This joint and its capsule have been identified as a source of neck pain, and a likely candidate for injury, in both clinical and biomechanical studies (Barnsley et al., 1993, 1994; Cavanaugh, 2000; Chen et al., 2005; Kallakuri et al., 1999; Lee et al., 2004a, b; Lord et al., 1996; Lu et al., 2005a, b). Engineering studies of the cervical facet joint have quantified excessive bony motions across the joint which can apply direct tensile loading to the facet capsular ligament (Bogduk and Yoganandan, 2001; Cusick et al., 2001; Deng et al., 2000; Pearson et al., 2004; Stemper et al., 2005). Kinematic studies of volunteers, whole spine and isolated cadaveric specimens have demonstrated that the facet capsule is at risk for excessive tensile strains and subcatastrophic injury during simulations of low-velocity impacts, further implicating its potential for injury during whiplash (Grauer et al., 1997; Ito et al. 2004; Kaneoka et al., 1999; Luan et al., 2000; Ono et al., 1997; Panjabi et al., 1998; Pearson et al., 2004; Sundararajan et al., 2004; Yoganandan and Pintar, 1997). Characterization of facet joint kinematics can test the hypothesis that altered joint mechanics contribute to capsule injury, but it does not directly define ligament behavior or identify a mechanism by which painful ligament injury may occur.

More recent cadaveric studies have quantified the cervical facet capsule strain field in isolated motion segment experiments. These studies demonstrated that excessive loading of the cervical spine can induce facet capsule stretch and potentially create minor ligament ruptures at conditions below the mechanical thresholds for gross failure of the ligament (Siegmund et al., 2001; Winkelstein et al., 1999, 2000). Subfailure injury to soft tissue has been shown to alter the overall mechanical function by producing laxity, decreasing stiffness, altering viscoelastic parameters and extending the toe region of stress-strain curves (Panjabi et al., 1996, 1999; Pollack et al., 2000; Provenzano et al., 2002). Evidence of minor ruptures, a large toe region, and regional variation in the strain field suggest that collagen fibers in the facet capsular ligament may undergo varied fiber recruitment or possess variable fiber alignment. However, no study has investigated the distribution of collagen fibers in the cervical facet capsule, or the regional alignment of these fibers in response to loading. Defining the cervical facet capsular ligament's fiber kinematics and its relationship to structural mechanics could help understand potential mechanisms of this ligament's injury.

Different imaging modalities have been used extensively to quantify fiber organization, kinematics, and orientation for many different types of soft tissues and loading conditions (Billar and Sacks, 1997; Dickey et al., 1998; Guerin and Elliott, 2005; Hansen et al., 2002; Martin et al., 1996; Provenzano et al., 2002; Thomopoulos et al., 2003; Thorton et al., 2002; Tower et al., 2002; Tower and Tranquillo, 2001a; Whittaker and Canham, 1991). More recent efforts have defined sub-cellular organization for hard and soft tissues (Yeh et al., 2004). Imaging approaches include polarization sensitive optical coherence tomography, small angle light scattering, magnetic resonance imaging, Fourier analysis and quantitative polarized light microscopy. These techniques have different optimal applications depending on the tissue-type, sample size and experimental test set-up (Dickey et al., 1998; Guerin and Elliott, 2005; Martin et al., 1996; Tower et al., 2002). For facet capsular ligament tissue, quantitative polarized light imaging (QPLI) offers a pragmatic method for measuring collagen orientation (Tower and Tranquillo, 2001a, b; Tower et al., 2002). The method is nondestructive, fast, affordable, and easy to implement for a thin sheet-like tissue such as the facet capsule. QPLI utilizes polarized light to exploit collagen's naturally birefringent properties (Vilarta and Vidal Bde, 1989). The mean collagen fiber direction and its alignment through the tissue thickness (i.e. retardation of light) can be determined by analyzing changes in the polarization of light after it has passesd through the soft tissue.

Thus, the goal of this study was to develop a QPLI system based on the work of Tower et al. (2002) to determine collagen fiber alignment in the human facet capsular ligament, and further, to implement it during tensile loading of the joint. The development of this system will allow a characterization of the relationship between ligament fiber responses and structural mechanics. Also, by identifying regional variability in the ligament fiber alignment at rest and during loading it may be possible to explore the potential mechanisms of painful joint injury below gross failure.

METHODS

A QPLI system was developed around an Instron testing machine. Three C6/C7 facet joints from two fresh-frozen unembalmed female post-mortem human subjects (ages 54 and 65) were tested in pure tension as a preliminary investigation into facet capsule ligament fiber kinematics.

Specimen Preparation and Loading Procedure

All musculature was cleared from the cervical spine specimens and C6-C7 facet joints (n=3) were removed *en bloc*. Any remaining musculature or tendon insertions on the surface of the facet capsule were removed by fine dissection. The ligamentum flavum was transected, and portions of the superior and inferior articular processes were removed to allow light transmission through the ligament (Figure 1). A pair of perpendicular Kirschner wires was inserted into both the superior articular process of C6 and the inferior articular process of C7. The bony ends of the specimen were then placed in testing cups mounted to an Instron 5865 machine (Instron Corp., Norwood, MA) and cast in dental stone (Figure 1). The specimen was re-hydrated with saline and preconditioned with 30 cycles of 1 mm tensile distraction. After 2 minutes of rest, the ligament was distracted in pure tension at 0.5 mm/s to 15 mm, which was sufficiently beyond gross failure. Load and displacement data were collected at 1 kHz.

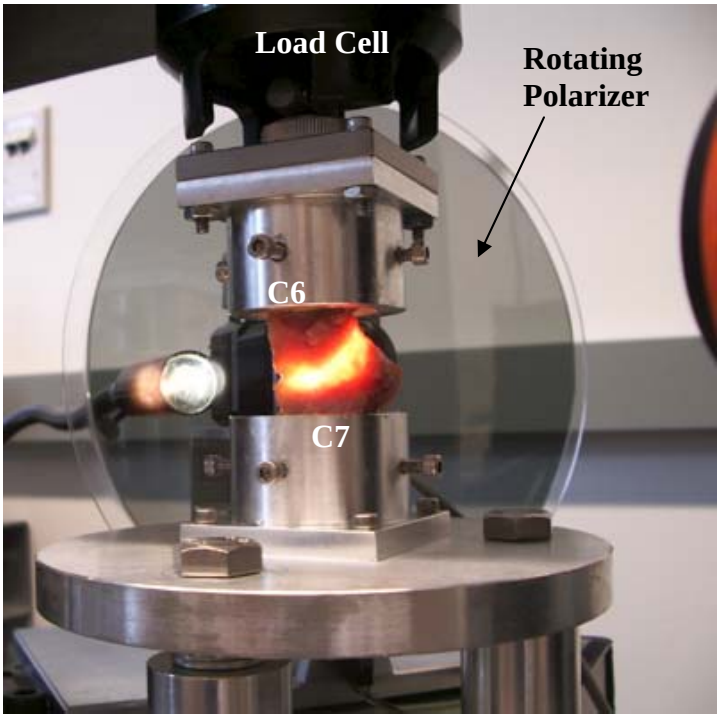

Figure 1: Right C6/C7 facet joint mounted to Instron with joint space illuminated by focused light source. Behind the specimen and test device is the rotating polarizer and focused light source.

Quantitative Polarized Light Imaging System

The QPLI system was constructed to operate with the Instron testing machine and acquire fiber data simultaneously. For this system, a NEMA 17 stepper motor, driver and controller (Lin Engineering, Santa Clara, CA) rotate an 8-inch cast acrylic disk with a linear polarizing laminated film (Edmund Optics Inc., Barrington, NJ). A fiber optic illuminator with focusing lens (Edmund Optics Inc., Barrington, NJ) is placed behind the rotating polarizer and transmits light through the ligament (Figures 1 and 2). As the light beam first passes through the rotating polarizer, it becomes linearly polarized at an angle dependent on the rotation of the polarizer. Then, as this linear polarized light travels through the birefringent ligament tissue, it is split into two orthogonal rays as it undergoes a net phase retardation, thus becoming elliptically polarized. The phase and amplitude of the elliptically polarized light is dependent on the direction of the collagen fibers in the ligament and the angle of the rotating polarizer. To analyze this elliptically polarized light, a circular analyzer was constructed using a Mica quarter-waveplate (Optosigma Corp., Santa Ana, CA) and linear polarizing film. This circular analyzer is mounted to a 6X macro zoom lens and CCD camera (Vision Research Inc., Wayne, NJ). As the elliptically polarized light enters the circular analyzer, it recombines the orthogonal light rays, and a CCD camera stores an 8-bit measure of light intensity for each pixel in the area of the ligament that is imaged. This measure of light intensity is dependent on both the fiber alignment in the ligament and the rotating polarizer angle.

For this study, the rotation of the polarizer was monitored by an optical sensor which was triggered every 180˚ of polarizer rotation. The data from the optical sensor were collected along with the load and displacement data by the Bluehill software package (Instron Corp., Norwood, MA), and were used to trigger and synchronize the CCD cameras with the structural mechanical data. A second CCD camera was used to verify the position of the rotating polarizer.

The CCD cameras collected images at 250 fps with 10 pixel/mm resolution. The polarizer was calibrated to rotate at 375 rpm, which corresponded to a 9˚ increment between each image acquired. Thus, a set of 20 raw images were acquired every 0.08 seconds as the polarizer rotated from 0 to 180˚. Each set of 20 images was used to create a single fiber alignment map. Given the acquisition rate, camera resolution, and the ligament distraction rate, ligament translation during the acquisition of a single alignment map was less than 0.4 pixels. As a result, continuous joint distraction was possible during the acquisition of fiber information.

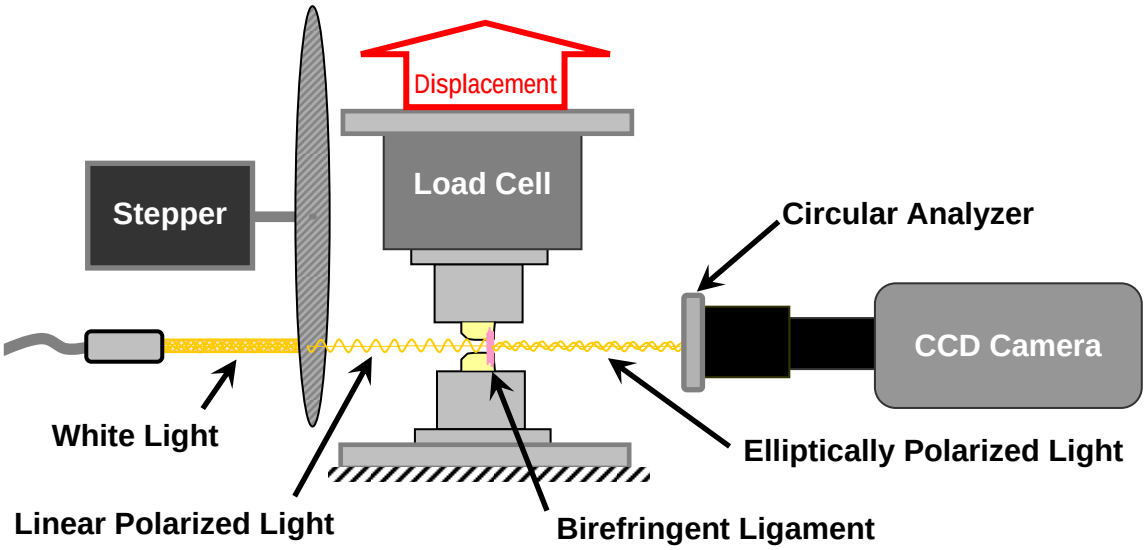

Figure 2: Schematic of the QPLI system.

Data Analysis

Harmonic analysis was employed to produce alignment maps for every set of 20 raw camera images (Tower et al., 2002). The intensity of each pixel in any set of consecutive images is described by the simple harmonic relationship:

$$I(\theta_i) = A + B\cos(2\theta_i) + C\sin(2\theta_i)$$

where θ_i is the rotation angle with respect to horizontal at each interval, *i*. *A* is the mean intensity, and *B* and *C* are the signed harmonic amplitudes. These Fourier coefficients *A*, *B*, and *C* were determined using a summation approximation (Tower and Tranquillo, 2001b; Tower et al., 2002). The coefficients were then scaled by dividing by:

$$\sqrt{B_0^2 + C_0^2}\left(\frac{A - dc}{A_0 - dc}\right)$$

where A_0, B_0, and C_0 are the harmonic coefficients from the QPLI system with a fixed linear analyzer rather than a circular analyzer, representing the system's maximum possible harmonic amplitude. The intensity offset, *dc*, corrects for background illumination and dark current. Once scaled, the coefficients *B* and *C* were used to calculate the alignment direction (α) and the retardation (δ):

$$\alpha = \frac{1}{2}\tan^{-1}(B/-C)$$

$$\delta = \cos^{-1}\left(\sqrt{1 - B^2 - C^2}\right)$$

However, most circular analyzers are not always optimized for a particular testing environment. Misalignment or the retardation of light outside its calibrated wavelength could result in an effective elliptical analyzer. To correct for these problems, the alignment and retardation of the quarter-waveplate were experimentally determined and the normalized Stokes polarization coefficients were calculated and used to correct the alignment and retardation measurements (Tower et al., 2002). Also, data from pixels that contained peak-to-peak amplitudes of less than 4 based on a 0-255 scale were removed from analysis to prevent noise at low intensities from creating false direction and retardation information.

Collagen fiber alignment maps of the facet capsular ligament were created for every 40 μm of distraction. For each alignment map, the mean direction and circular variance were calculated using circular statistics. The mean retardation and its X-(horizontal) and Y-(direction of loading) components were calculated. These measures of the mean fiber behavior were compared to the force response during distraction.

RESULTS

Gross failure of the ligament occurred at 66.3±11.1 N and 5.84±1.81 mm. Between 0 and 1 mm of distraction, light transmission was limited, and as a result fiber information was very sparse. In general, considerable changes in the mean direction, directional variance, and retardation occurred prior to failure in all specimens. For distractions below gross failure but above 1 mm, retardation and circular variance decreased for all specimens. Fiber alignment was most dominant in the direction of loading at all distraction magnitudes.

All specimens demonstrated acute changes in alignment prior to failure. A single representative specimen is presented here for simplicity. The fiber directions in this representative ligament sample demonstrate spatial variability in the alignment, particularly below 3 mm of distraction (Figure 3). This spatial variability in direction, however, typically decreased with increasing distraction (Figure 3), resulting in an overall mean direction that was 15˚ to 19˚ off from the direction of loading before failure (Figure 4). Interestingly, the retardation, which is a measure of the strength of the fiber alignment through the thickness of the tissue (Z-direction) at any given pixel, appeared to decrease in some regions with increases in distraction (Figure 3).

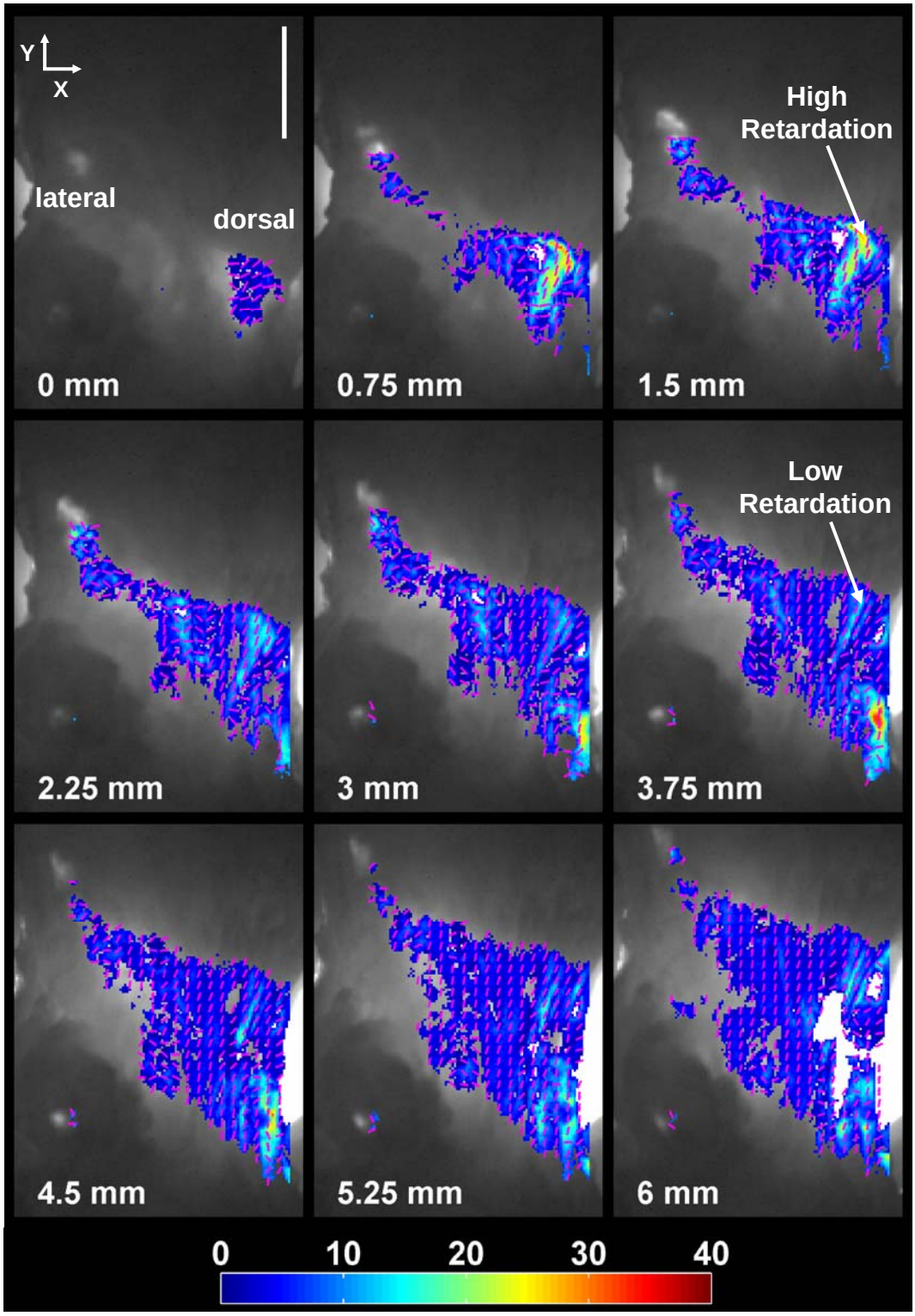

Figure 3: Fiber alignment maps of a left C6/C7 facet capsule (#C893-C67L) at 0.75 mm increments of applied distraction. Distraction was in the Y-direction, as defined by the axes in the first frame (0 mm). For ease of visualization, each overlaid line (magenta) represents the mean fiber direction of a 5x5 pixel region. The underlying color map represents the strength of alignment with high retardation (red) representing highly aligned regions (see scale at bottom). The arrows note a region of decreasing retardation. The calibration bar (first frame) equals 5 mm.

Mean fiber direction changed as distraction increased, with an initial change in alignment directed towards the loading axis, followed by a reversal of direction at 2.07 mm (Figure 4). The mean fiber direction of this specimen (#C893-C67L) ranged between 71˚ and 80˚ (Figure 4). Like the alignment maps, mean directional variance demonstrated an increase in overall alignment (i.e. decrease in variance) beyond the first 1 mm of distraction (Figure 5). The decrease in variance was most noticeable between 1.5 and 2 mm. Except when light transmission was limited between 0 and 1 mm, mean retardation tended to decrease with increasing distraction (Figure 6). The decrease in retardation was most pronounced between 1.9 and 2.1 mm (Figure 6). Retardation in the Y-direction was consistently more dominant than the X-direction throughout the loading paradigm.

Preliminary efforts were carried out to characterize the error of this system. Inaccuracies in measuring retardation and direction with this QPLI system were identified. Due to its birefringent properties, a thin PTFE strip was placed at a known angle (0˚), and fiber direction information was measured with the QPLI system for a 100x100 pixel area. The mean difference between the known and measured direction was calculated as 2.93±1.45˚. Error in the retardation measurements was estimated by acquiring data without a sample (0˚ of retardation); the mean retardation error was 0.85±0.44˚. Given the range of experimental values (0-180˚ for direction, 0-40˚ for retardation), this degree of error in the system was considered acceptable. Quarter waveplate misalignment in the circular analyzer was measured as 1.34˚ and the mean light retardation was measured as 90.64˚, both of which were consistent with or an improvement over previous studies (Tower et al., 2002).

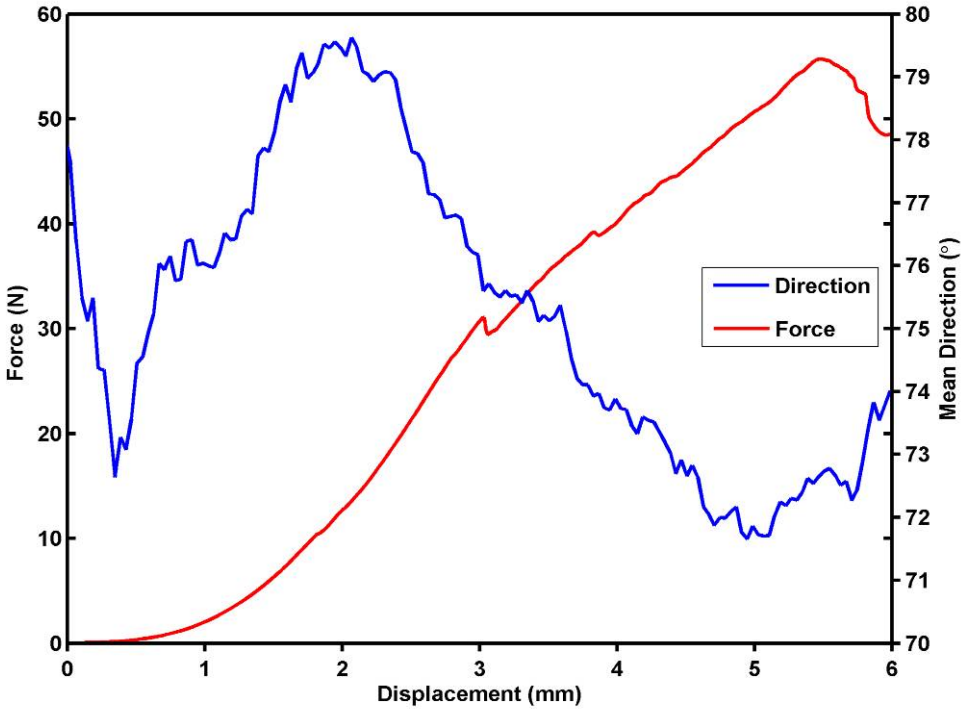

Figure 4: Force and mean alignment direction during distraction for specimen #C893-C67L. Direction is measured with respect to the positive X-axis, so the direction of loading was 90˚ (along Y-axis).

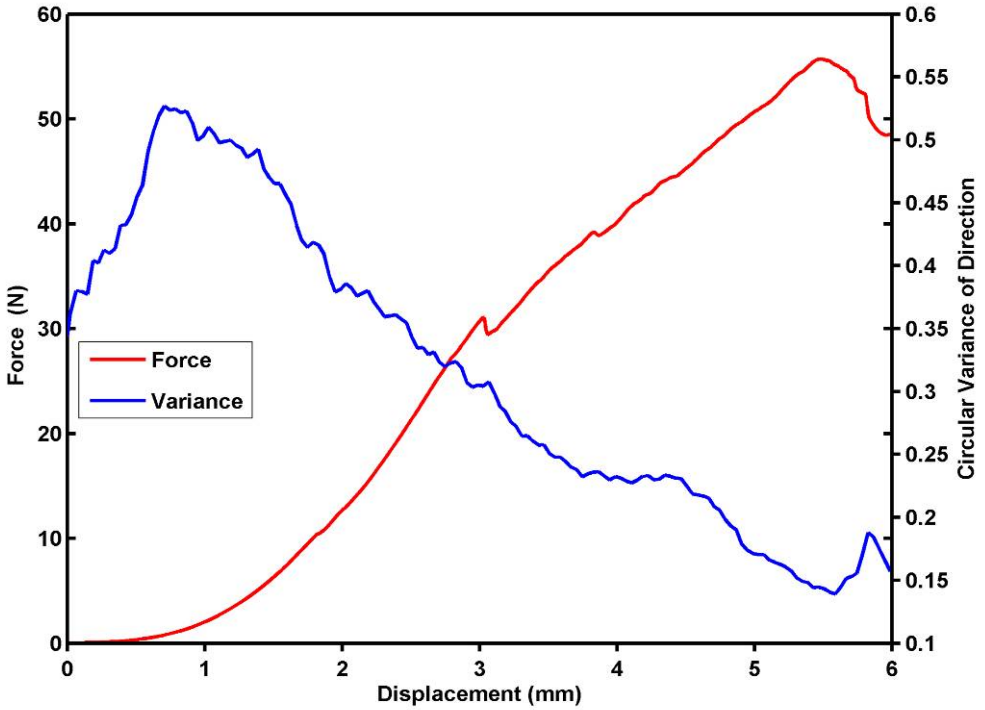

Figure 5: Force and directional variance during distraction for specimen #C893-C67L. The decrease in variance with increasing displacement indicates increasing alignment in the X- and Y- directions.

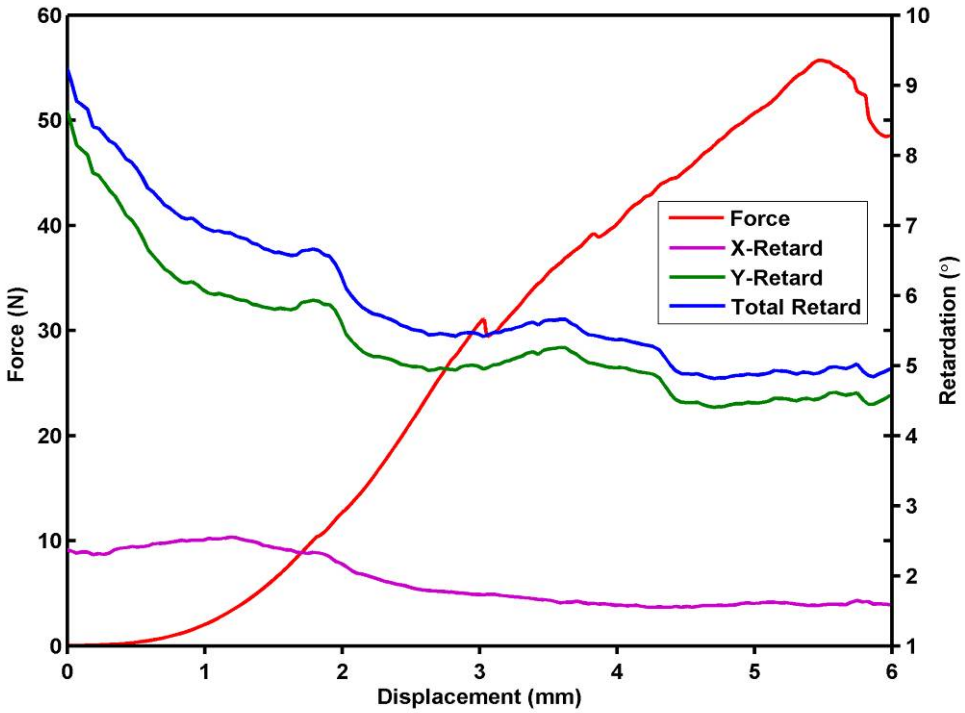

Figure 6: Force and mean retardation during distraction for specimen #C893-C67L. The X and Y components of mean retardation (magenta & green, respectively) are shown, as well as mean total retardation (blue). The decrease in retardation with increasing distraction indicates the decreasing mean alignment through the thickness of the ligament (Z-direction).

CONCLUSIONS

While previous studies have demonstrated this QPLI technique to be capable of defining fiber alignment (Tower et al., 2002, Tower and Tranquillo, 2001a, b), this is its first application for the facet capsular ligament during tensile loading. The load and displacement at which gross failure occurred (66.3 N, 5.84 mm) were similar to previous quasistatic failure tests of the human facet capsular ligament in pure tension (Winkelstein et al., 1999, 2000), suggesting that the sample preparation required for detecting the dynamic fiber response in this approach does not alter the ligament's intrinsic structural response. Further, these pilot results suggest a complex pattern of fiber alignment that undergoes structural changes prior to gross failure.

The decrease in retardation (Figure 6) also suggests a decrease in the strength of fiber alignment through the thickness of this ligament (the Z-direction in Figure 3) with increasing joint distraction. This is an unexpected result, because the strength of alignment increases in the X- and Y-directions with increasing distraction (Figures 3 & 5). This anisotropy in the dynamic fiber response may be the result of the dissociation or delamination of distinct sheets of fibers. However, histological examinations are required to verify this hypothesis.

Considerable changes in these alignment measurements occurred between 1.5 and 2 mm of distraction in the specimen shown (Figures 4-6). Surprisingly, all of these optically measured structural changes occurred prior to minor rupture (3.03 mm) and gross failure (5.48 mm) for that specimen. These same trends were found in the other specimens, as well. However, a recent study of the rat facet capsule demonstrated ligament yield, as defined by a decrease in stiffness, to occur at a significantly lower distraction than gross failure (Quinn and Winkelstein, 2007). By differentiating the force-displacement curve using a centered finite difference approximation, a measure of instantaneous stiffness can be obtained for this sample. When stiffness is plotted with retardation, a sharp decrease in stiffness (i.e. yield) is noted beginning at 1.8 mm of distraction (Figure 7). Given the structural changes detected by the QPLI system near yield, further studies are warranted to investigate the potential of QPLI in detecting the location and threshold of yield and structural damage.

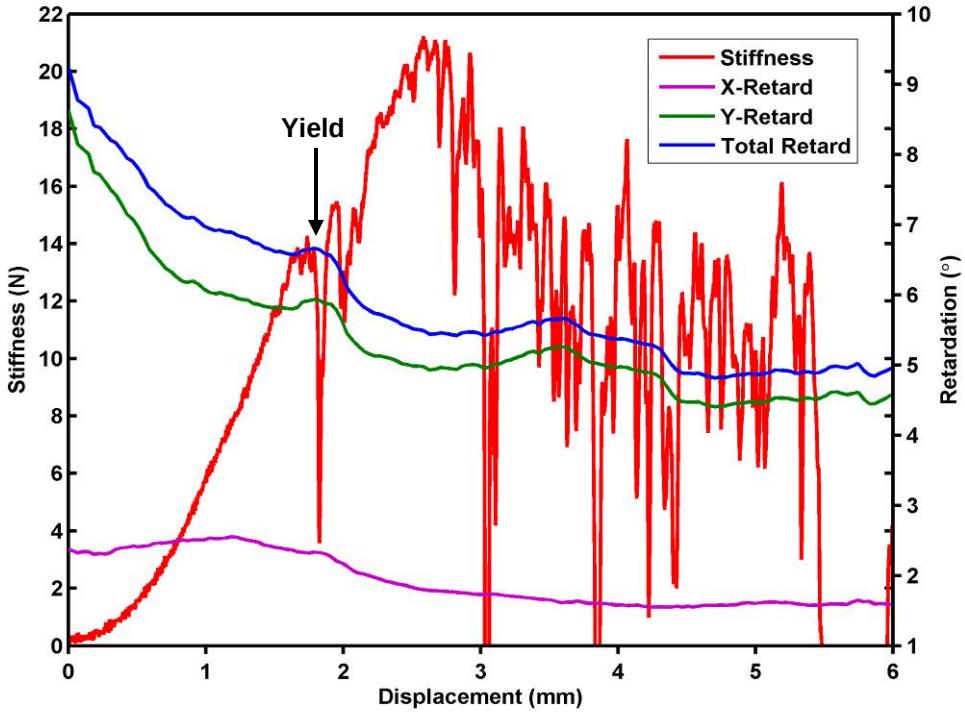

Figure 7: Stiffness and mean retardation during distraction for specimen #C893-C67L. X- and Y-components of mean retardation (magenta & green, respectively) are shown, as well as total mean retardation (blue). The ligament yield point is indicated by an arrow; yield may correspond with changes in retardation.

A number of challenges exist with implementing this imaging technique for the human cervical facet capsule. Transection of the ligamentum flavum prior to casting enabled bone removal, but may change the unloaded reference position of the ligament. As a result, multiple reference position measurements are needed to ensure a physiologic reference position and consistent loading paradigm. A minimum of two length measurements (one on the dorsal aspect, another on the lateral) prior to transection is required to ascertain relevant mechanical thresholds. However, in this study, reference was defined by a 0.01N preload. Additionally, special care must be taken in removing muscle and tendon insertions on the outer surface of the ligament. The birefringence of these tissues can skew the ligament's directional and retardation data because they are acquired through light transmission and the data through the thickness are combined. Variation in measured retardation can also arise from changes in the background illumination and ligament thickness. The QPLI system cannot directly measure thickness so spatial or temporal variations in capsule geometry can directly affect retardation measurements. A measure of the contribution of regional thickness to variation in retardation should be made. Finally, in the unloaded state, bone will obstruct much of the light transmission and limit the amount of data spatially that can be acquired, particularly at low levels of distraction (Figure 3). Excision of the ligament may be required in future studies to assess alignment in the unloaded or nearly unloaded state.

The description of ligament fiber kinematics and re-orientation through QPLI techniques presented here offers a potentially useful approach to defining the microstructural mechanisms of local injury. Many studies allude to subfailure loading of the facet capsular ligament as a mechanism of injury (Chen et al., 2005; Ito et al., 2004; Lu et al., 2005a, b; Siegmund et al., 2001; Winkelstein et al., 1999, 2000), but no work has specifically examined the microstructural response of this ligament in the context of pain and injury. Such investigations would, for the first time, establish a mechanism of injury and identify potential strategies for prevention or repair.

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DISCUSSION

PAPER: **Application of Quantitative Polarized Light Techniques for Characterizing Ligament Fiber Kinematics during Facet Joint Loading**

PRESENTER: ***Kyle Quinn, Department of Bioengineering -- University of Pennsylvania***

QUESTION: *John Cavanaugh, Wayne State*
Nice work. Do you have any plans, during this process as you load the tissue and you see these changes in direction and stiffness, to stop the loading and take the tissue out and perhaps do some histological studies to see if there's any correlation between what you might find under a microscope and your changes?

ANSWER: Yeah. We definitely would like to dynamically assess any changes in stiffness and then potentially stop it. And definitely, we'll get that. So, yeah.

Q: Thank you.

Q: *Guy Nusholtz, Daimler Chrysler*
What effects do these observations have on the bulk properties of the ligaments that you're looking at?

A: Well, I mean it certainly indicates that the ligament's anisotropic and you know, we hope that this data will somehow allow us to develop a more biofidelic model of the joint.

Q: Right. But if it's, you know—Assuming that the response is elastic, you know the complexity of what's going on. But in terms of the bulk process—what we might use in, say, a model, it would generally be the same unless you're showing a very complicated response to loading across the ligament. Or, the other option is that it mimics, say, elastic plastic; but in reality, there's no damage being done, then that would have an implication for the injury profile.

A: Yeah, yeah, definitely. We'll definitely look into that more.

Q: Thank you.