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Fiber Orientation of the Transverse Carpal Ligament

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Abstract

The transverse carpal ligament is the volar roof of the carpal tunnel. Gross observation shows that the ligament appears to have fibers that roughly orient in the transverse direction. A closer anatomical examination shows that the ligament also has oblique fibers. Knowledge of the fiber orientation of the transverse carpal ligament is valuable for further understanding the ligament's role in regulating the structural function of the carpal tunnel. The purpose of this study is to quantify collagen fiber orientation within the transverse carpal ligament using the small angle light scattering technique. Eight transverse carpal ligament samples from cadaver hands were used in this study. Individual 20 μ m sections were cut evenly along the thickness of the transverse carpal ligament. Sections of three thickness levels (25%, 50%, and 75% from the volar surface) were collected for each transverse carpal ligament. Fibers were grouped in the following orientation ranges: transverse, longitudinal, oblique in the pisiform-trapezium (PT), and oblique in the scaphoid-hamate (SH) directions. In analyzing the fiber percentages, the orientation types for the different thickness levels of the ligament showed that the transverse fibers were the most prominent (>60.7%) followed by the PT oblique (18.6%), SH oblique (13.0%), and longitudinal (8.6%) fibers.

Keywords

Transverse carpal ligament; Collagen fiber orientation; Small angle light scattering

Introduction

The volar side of the carpal tunnel is formed by soft tissues, which is commonly referred to as the flexor retinaculum (Cobb et al. 1993). The flexor retinaculum consists of three portions: the proximal thin antebrachial fascia, the middle thick transverse carpal ligament

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Competing interests

There are no competing interests in this study.

(TCL), and the most distal aponeurosis between the thenar and hypothenar musculature (Cobb et al. 1993; Brooks et al. 2003; Pacek et al. 2010). Recent studies of the flexor retinaculum suggest that the TCL be a preferred term to delineate this tissue structure because the TCL has specific anatomy for its distinct fibrous lamina and bony insertions (Pacek et al. 2010; Stecco et al. 2010). The TCL attaches ulnarly to the pisiform and radially to the tubercle of the scaphoid for its proximal end while connecting to the hook of hamate on the ulnar side and to the ridge of trapezium on the radial side for its distal attachments. Biomechanically, the TCL serves as a stabilizer for the carpal tunnel along with the thenar and hypothenar muscles while forming a pulley for flexor tendons (Fuss and Wagner 1996; Brooks et al. 2003; Stecco et al. 2010).

Current work on the TCL has been scarce and limited. Morphology of the TCL has been studied in the past to understand its structure in terms of thickness and proximal-distal length (Pacek et al. 2010). General and experimental observations have also shown the TCL to have a dense and inextensible collagenous matrix with a predominant alignment in the transverse direction (Rotman and Donovan 2002; Stecco et al. 2010). Other studies have determined the various fiber configurations of the TCL. Two configurations have shown to be the most prominent with both arrangements having transverse and oblique fiber variations (Isogai et al. 2002). Our current knowledge on the fiber orientation of the TCL is rather rudimentary.

The small angle light scattering (SALS) technique was developed to map the preferential directions of the fiber structure within soft tissues (*Sacks et al. 1997*). It has been applied to quantify fiber orientation of heart valves (*Sacks and Schoen 2002*), cornea (*McCally and Farrell 1999*), cranial dura mater (*Hamann et al. 1998*), glenohumeral ligament (*Debski et al. 2003*), and remodelling of collagen fiber alignment (*Nguyen et al. 2009*). The purpose of this study was to use this SALS technique to characterize the orientation of the TCL's collagen fibers. We hypothesize that the overall majority of fibers would be transverse with some fibers orienting obliquely inserted either from the pisiform bone onto the trapezium or from the scaphoid onto the hamate.

Methods

Experimental Procedures

The process used to analyze the TCL was broken down into five phases: dissection, fixation, tissue sectioning, image preparation, and SALS imaging. The process took approximately four days to complete. Data processing and statistical analyses were additionally done to reveal preferential fiber orientation throughout the depth of each TCL.

The dissection and fixation stages took place on the first day. This process allowed for the removal of TCL to be preserved before slicing and imaging. Eight fresh frozen hand specimens (53 ± 13 years) were selected through review of their medical history record to exclude those found with muscular-skeletal injuries or surgeries to the hand and the wrist. The dissection aimed to remove the skin and soft tissue attached to the volar side of TCL. The TCL was identified as a dense, thick, and fibrous bundle of collagen. Further isolation was carried out through identifying its insertion sites on the carpal bones. This tissue was removed at its insertion sites. The transverse lengths of the TCL were 15.3 ± 4.9 mm at the distal (trapezium-hamate) level and 20.0 ± 4.3 mm at proximal (pisiform-scaphoid) levels.

Fixation of the tissue was performed immediately after its removal with its immersion in 10% phosphate buffered formalin for 24 hours. Then the tissue was frozen in -20°C temperature for another 24 hours. The embedding and slicing stages took place on the third day. Specimen thickness was measured with digital calipers while the tissue was still frozen.

The thickness of the tissue was 3.2 ± 0.8 mm. Two holes were punched out through the thicknesses at the midpoints of the proximal and distal ends to define a midline along the proximal-distal direction of the TCL. Each ligament was mounted in a medium set at optimum cutting temperature and placed within a cryostat (Microm®, Thermo-Scientific; Waltham, MA), which preserved the tissue at a temperature of -20°C . The cryostat was set to slice the specimen at $20\text{ }\mu\text{m}$ thicknesses. Sections of interest were obtained on glass microscope slides at 25% (volar), 50% (middle), and 75% (dorsal) of the TCL thickness (depth with 0% signifying its volar surface). All slides were kept within a specimen freezer set at -20°C until imaging. Each slide was placed in gluteraldehyde solution for 5 minutes. This solution increased the slide's transparency by dissolving any extraneous artifacts left on the slide. Slides were rinsed in distilled water and then left to dry. All slides were cleaned with individual wipes and glass cleaner.

Each slide was placed in the sample holder of the SALS apparatus to be individually scanned. The processing stage consisted of the imaging phase which consisted of several steps. SALS imaging has been utilized in previous works and its approach has been well established (*Sacks et al. 1997*). Briefly, an unpolarized 4 mW HeNe laser was directed on to the slide. The positioning of the light beam was centered on the camera's lens scan the total area of the tissue section. This was accomplished with the movement of the spatial filter-beam expander and the camera. Scattered light data was gathered and digitized using an image grabber board in conjunction with the camera to obtain the fiber network orientations across each tissue section.

Data Processing

Orientation data was accumulated for each scanned area in the form of light intensity distributed over angles covering one revolution. This area was equivalent to a circle with the diameter of $254\text{ }\mu\text{m}$. Each intensity distribution withholds two concerning variables: the preferred fiber direction and the orientation index (OI) (*Sacks et al. 1997*). The analysis of each slide led to an image which displayed these features for each scanned area spanning the whole tissue section.

The distribution centroid represented the overall preferred direction for the fibrous network located within each scanned area. Accuracy of the estimated preferred direction could be inferred from the difference between each light intensity distribution's centroid and its corresponding angle of peak intensity. A large difference indicated that a large skew was present. Possible reasoning behind this significant skew could be that many different fiber networks, which were aligned in multiple directions, overlaid each other within the concerning scanned area of the tissue section. In the case where a small skew was present, fiber networks were coincident in the measured preferred direction validating the centroid measurement. A simple verification for the preferred direction can incorporate the OI which determines the angle at which one-half of the total fibers are oriented. This is determined through the difference between the maximum and minimum angle of the range where one half of the total area of the light intensity distribution is situated. Highly oriented, scanned areas have low OI values while randomly oriented, fiber networks have large OI values (*Sacks et al. 1997*).

Several adjustments had to be made, in order to analyze the fiber orientations among each depth. The preferred directions had to be corrected to the proximal-distal midline of the TCL and artifacts had to be removed. The former was done based off of inspecting the two holes within each tissue section's resulting SALS image. Original preferred directions were subtracted by this value. Further efforts of removing cutting artifacts consisted of utilizing an OI value of 45 to isolate actual fiber orientations (*Debski et al. 2003*).

Data Processing

Histograms of the preferred directions for each depth were created to see if a pattern of fiber orientation existed. The frequency of preferred directions for each angle spanning 0°-180° was normalized with respect to the number of scanning areas within each tissue section. Normalized frequencies of all sections for each depth were summed across different specimen and averaged.

Four types of fiber orientations for each section were considered: Transverse (0°-22.5°, 157.5°-180°), Oblique in the pisiform-trapezium direction (PT Oblique, 22.5°-67.5°), Oblique in the scaphoid-hamate direction (SH Oblique, 112.5°-157.5°), and Longitudinal (67.5°-112.5°). See Figure 2(a). Percentages of the preferred directions for the four orientations were calculated within those ranges.

The Watson-William's test investigated the differences of fiber alignment with respect to thickness depth. Fiber alignment was quantified using the angular dispersion of preferred directions for each section (*Nguyen et al. 2009*). This measurement is equivalent to calculating the standard deviation of an array of data set on a circular scale. Overall, the analysis attempted to compare the spread of preferred directions of fiber networks among different depths. Groups were compared for the volar (25%), middle (50%), and dorsal (75%) slices. A two-factor mixed measures analysis of variance (ANOVA) was used for statistical analyses. In the case of a difference, a subsequent one-factor ANOVA was used. Additionally, if the data did not meet the prerequisite of normal distribution, the equivalent non-parametric, Friedman's ANOVA were used. Pairwise comparisons determined whether differences existed between each group. Significances were considered at $\alpha=0.05$ for all statistical analyses.

Results

A sample SALS output is shown in Figure 1 revealing the variables of the preferred directions and the OI. The green specks signify the angles of orientation for each scanned area, i.e. preferred directions. The color of the slice indicates the order of alignment or the OI. Red and purple areas signify aligned fiber networks while green and blue areas signify sparsely arrayed fiber networks directed over the area (Figure 1).

Figure 2(b) is a representative polar plot of the histogram distribution of fiber orientation at the middle (50%) slice level. The Watson-Williams test showed that fiber orientation didn't vary with respect to tissue level ($P > 0.25$). Non-parametric Friedman's ANOVA showed significant difference among the different types of fiber orientations (Figure 3; $P < 0.001$). Post-hoc testing for fiber percentages indicated that the transverse fibers were the predominant type of fiber, followed by oblique fibers and longitudinal fibers (Figures 2 and 3). Overall averages were the following: $60.7 \pm 13.7\%$ for the transverse fibers, $18.6 \pm 10.6\%$ for the PT Oblique fibers, $13.0 \pm 6.7\%$ for the SH Oblique fibers, and $8.6 \pm 5.1\%$ for the longitudinal fibers.

Discussion

The TCL was found to have a preferred degree of fiber alignment which suggested material anisotropy. The results showed that the predominant fiber direction was transverse while longitudinal and oblique fibers were marginal. These findings are supported by biomechanical testing of the TCL showing that the tangent modulus in the transverse direction was many times greater than that in the longitudinal direction (*Xiu et al. 2010a*). This anisotropic property should be considered for future modeling applications.

Results revealed that the fiber distribution did not change with respect to depth, but significant differences were found comparing across the different types of fiber orientations. Our data are consistent with the gross observation (Isogai et al. 2002) and staining methods (Stecco et al. 2010) with TCL fibers predominately aligned in the transverse direction. In an observational study, Isogai et al. (2002) reported that the fiber laminar configuration varied from specimen to specimen and categorized them into four configurations of fiber orientation. Type I configuration is distal transverse and ulnar (pisiform to trapezium) oblique in all laminae of the TCL; type II configuration is distal transverse and ulnar (pisiform to trapezium) oblique in the superficial layer, and proximal transverse and radial oblique (scaphoid to hamate) in the deep layer; type III configuration is distal transverse and ulnar oblique in the superficial layer and proximal/distal transverse in the deep layer, and type IV configuration is transverse at all depths with no clear oblique fibers. However, our data showed that the orientation pattern did not differ among the volar, middle and dorsal layers. With histological data, Stecco et al. (2010) showed that the flexor retinaculum has two types of fibrous structures – the superficial layer in continuity with the antebrachial fascia and the ligament-like layer one composed of strong lamina. Our results did not show the bi-laminar configuration. Likely, the tissue layer of antebrachial reinforcement was not represented in our study because the most superficial layer in the current study was at 25% depth from the volar surface. Therefore, uniform fiber distribution pattern is indicative of the structural properties of the TCL. Though our study provided more quantitative results of TCL fiber orientation, future studies using more slices covering the entire depth may help confirm the types of laminar configuration observed by Isogai et al. (2002) and Stecco et al. (2010).

Predominance of the transverse fiber orientation could play an important role in maintaining the carpal arch. This inference made us believe that a majority of transverse stability is provided by the TCL; whereas carpal arch expansion in the oblique or longitudinal directions may not be as important. Such directionality was also featured in measuring changes to carpal tunnel compliance before and after TCL transection (Garcia-Elias et al. 1989; Xiu et al. 2010a; Xiu et al. 2010b). In an unloaded state, the TCL fibers may display a wave form configuration (Stecco et al. 2010). This means that the same fiber may have different orientations at different points along its length. The orientation indices based on regional information may not accurately correlate with the amount of fibers in a certain direction. A possible improvement could be to load the TCL sample during the tissue fixation.

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Figure 1.
A Sample SALS output of color map and vectors indicating the degree of alignment and preferred direction

Figure 2. Definition of the fiber orientations (a) and orientation histogram at 50% level (b) in the transverse, pisiformtrapezium (PT Oblique), longitudinal, and scaphoid-hamate (SH Oblique) directions

Figure 3.
Fiber percentages measured with respect to slice depth