RANDOM COLLAGEN FIBER ARCHITECTURE IN THE AXILLARY POUCH OF THE INFERIOR GLENOHUMERAL LIGAMENT: EFFECT ON CAPSULAR SHIFT PROCEDURES

INTRODUCTION
The glenohumeral (GH) joint is the most commonly dislocated joint in the body, with recurrence following open and arthroscopic surgical repair as high as 12% and 23%, respectively. [1] Anterior dislocation results in injury of the anteroinferior capsule-labral structures which include the inferior glenohumeral ligament (IGHL), the primary static restraint to anterior joint dislocation. [2] The organization of the collagen fibers in this tissue could yield information about its in vivo function. Additionally, it may improve surgical repair techniques as axillary pouch injury may be superior-to-inferior as well as medial-to-lateral. [3] The collagen fibers of the anterior band (AB-IGHL) were previously reported to be oriented parallel to its long axis but the collagen fiber orientation of the axillary pouch remains uncertain. [4,5] The purpose of this study was to quantify the collagen fiber orientation in the axillary pouch using the small angle light scattering (SALS) technique. We hypothesize that the collagen fibers in the axillary pouch of the IGHL are randomly oriented throughout the thickness of the tissue.

MATERIALS AND METHODS
Eight fresh frozen human cadaveric specimens (avg. age 50.8±8.6 years) were dissected. Six specimens were utilized in preliminary investigations to obtain a satisfactory method for acquiring and maintaining histological samples as well as establish a method to quantify the fiber orientation using polarized light microscopy. However, due to the randomness of the fibers and subjectiveness of the results, polarized light microscopy was not an effective technique to obtain quantitative data.

Three rectangular samples (approx. 11 x 6 mm) were excised from the axillary pouch of the two remaining specimens. Each sample was harvested with one edge parallel to the longitudinal axis of the AB-IGHL. Samples of the long head of the biceps tendon (LHBT) were also obtained as a highly aligned control. Each sample was immersed in a beaker of 2-methyl butane, which was surrounded by liquid nitrogen. Once frozen, the samples were stored at –80°C until sliced on a cryostat at 100 µm increments. Approximately 10 slices per sample were collected, allowing the variability of collagen fiber alignment to be compared throughout the depth of the tissue. The samples were then mounted on slides and kept in a cold room (5°C) until tested within 24 hours.

Previous studies have utilized the birefringent optical properties of collagen in order to quantify the orientation of fibrous tissue using SALS. [6] The SALS device passes a 4 mW unpolarized HeNe laser beam, chosen because its wavelength (632.8 nm) is within an order of magnitude of the collagen fibril diameter, through the tissue. [6] When the laser passes through the tissue, light is scattered perpendicular to each fiber axis, thus producing a scattered light intensity. The maximum intensity is achieved at the angle of greatest alignment of the collagen fibers.

Each slide was placed individually on the SALS machine and the collagen fiber orientation was measured. The SALS device has previously been determined to have a spatial resolution of ±254 µm and the ability to quantify the predominant collagen orientation to within 1°. [6] An orientation index (OI) can be defined as the angle containing 50% of the total number of fibers. An increase in OI is directly related to an increase in randomness. At three depths within each sample, the distribution of OI values in a 9.3 mm² area was calculated and compared to the distribution of OI values in an area with the same dimensions for a slice of LHBT. [6]

RESULTS
The collagen fibers of the axillary pouch appeared to be randomly oriented for each slice and also throughout the thickness. Compared to the OI distribution of the highly aligned LHBT (Fig.1), the OI distributions of one representative sample of axillary pouch tissue are clearly more random (Figs. 2,3,4). The darker regions represent regions with higher OI values, and therefore regions of less organization. The predominant direction of collagen fiber alignment is shown by the black lines in the OI distribution figures. Within the axillary pouch tissue, there appear to be small regions of moderate organization within a largely unorganized tissue, as shown by the darker appearance of the axillary pouch tissue (Figs. 2, 3, and 4) compared to the lighter appearance of the LHBT (Fig.1). The OI distributions show the lack of collagen fiber organization in individual slices and also throughout the depth of each sample. Averaging the results from both specimens, 45.3±9.6% of the area of the LHBT slices had an OI of 41 degrees or less, compared to the axillary pouch tissue slices which had 23.0±6.9% of the area with an OI of less than 41 degrees. The randomness of the tissue throughout the thickness was shown by comparing the percentage of area with an OI of less than 41 from slices near the bursal (24.4±9.4%), middle (23.5±5.1%), and articular (20.9±6.4%) portions of the capsule.

DISCUSSION
The results obtained from SALS support our hypothesis that the resultant fiber architecture of the axillary pouch showed the collagen fiber orientation to be random throughout the thickness of the tissue. Although some regions of localized alignment were noted, no evidence of a pattern for an individual depth or throughout the thickness was present. Our findings disagree with previous studies regarding fiber orientation in the axillary pouch that were obtained using polarized light microscopy. [4,5] As expected, the LHBT demonstrated a high degree of alignment with respect to its longitudinal axis. However, due to its lack of collagen fiber alignment, the axillary pouch does not appear to have the morphological characteristics of a ligament.

This random fiber orientation will affect the mechanical properties of the axillary pouch and implies that they would be similar in the directions parallel and perpendicular to the capsular ligaments. This also suggests that capsular shift procedures should treat all components of the IGHL as a continuous sheet. Specifically, proper fixation to the rim of the glenoid, in both the medial-lateral and superior-inferior direction, is necessary to restore intact capsular function.

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REFERENCES

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