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Is the Circumferential Tensile Modulus within a Human Medial Meniscus Affected by the Test Sample Location and Cross-Sectional Area?

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Summary: Quantifying the material properties of the human menisci is paramount to understanding their biomechanical functions within the knee. One important intrinsic material property governing the biomechanical functions of the meniscus is the circumferential tensile modulus. The purpose of this study was to determine if the circumferential tensile modulus of the human medial meniscus depends on the location and thickness of the sample tested. The following three hypotheses were tested: (a) the circumferential location (anterior, central, and posterior) does not significantly affect the tensile modulus, (b) the radial location (inner to outer) significantly affects the tensile modulus, and (c) the thickness (cross-sectional area) significantly affects the tensile modulus. Test samples, whose length was oriented in parallel with the circumferential collagen fibers, were collected from different circumferential and radial locations throughout 30 human medial menisci. Samples of three different thicknesses (0.5, 1.5, and 3.0 mm) were taken from three equal groups of 10 menisci (i.e., one thickness per group). The circumferential tensile modulus was measured under quasi-static loading. Statistical analysis showed no significant effect of the circumferential or radial location of the sample on the circumferential tensile modulus. This indicates that an overall circumferential tensile modulus may be calculated for the human medial meniscus by averaging the values determined at the various locations. However, the thickness of the test sample had a significant effect on the measured circumferential tensile modulus; the modulus varied inversely with the thickness. Thus, moduli determined from test samples that are too small in cross-sectional area overestimate the effective modulus of the tissue on the whole, and the cross-sectional area of the sample must be considered when determining a representative circumferential tensile modulus for the medial meniscus in a human knee.

Knowledge of the material properties of the human meniscus is important to the study of injury mechanics (10), injury repair (11), and tibiofemoral contact mechanics (19,20). Mathematical models have studied the role of the meniscus in the load transmission of the tibiofemoral joint and concluded that the circumferential tensile modulus is one important material property (19,20). If the tensile modulus varies along the same circumferential line between regions (anterior, central, and posterior) or between different radial locations (inner to outer edge) within the same region, then this variability would have to be quantified to gain a complete understanding of the material behavior of the meniscus and would have to be included in finite element models.

Assuming that the distribution of circumferential collagen fibers within the meniscus determines the circumferential tensile modulus, this modulus would be expected to vary between radial locations but not between circumferential locations because of the way that the collagen is distributed. In the ultrastructure, the concentration of collagen fiber bundles is greater in the peripheral two-thirds than the inner third of the meniscus (7,15); therefore, the tensile modulus may vary between radial locations and be greater toward the periphery than the inner edge. However, the circumferential collagen fiber bundles are continuous from the anterior to the posterior horns (11), indicating that the tensile modulus should not vary between circumferential locations at a specified radial location.

The variability in the tensile modulus between circumferential locations has been investigated in other studies, but the findings were conflicting. Fithian et al. (6) reported that the anterior region of the medial meniscus had a greater tensile modulus than the central or posterior regions. Conversely, Tissakht and Ahmed (21) found no significant difference in the tensile modulus between regions. Neither study determined whether the tensile modulus varied radially. To deter-

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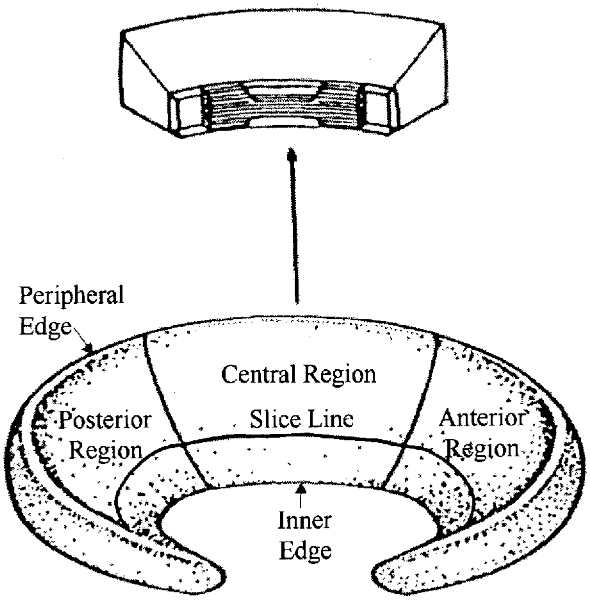

FIG. 1. Diagram indicating the circumferential and radial locations of the test samples within the medial meniscus. The first slice from which samples were taken was 2-3 mm from the inner edge, and contiguous slices were collected to the peripheral surface.

mine if the circumferential tensile modulus of the human medial meniscus varies regionally or radially, or both, the following two hypotheses were tested: the circumferential location (anterior, central, and posterior) does not significantly affect the tensile modulus whereas the radial location (inner to outer) does. Testing these two hypotheses was important to gaining a complete description of the tensile behavior of the meniscal tissue.

Another inconsistency between studies was the average value reported for the circumferential tensile modulus. Fithian et al. (6) noted that the anterior region of the medial meniscus had a tensile modulus of 159.6 MPa, which was 50% greater than the 106.2 MPa tensile modulus reported by Tissakht and Ahmed (21). This discrepancy may have been related to differences in the cross-sectional area of the tissue samples. The cross-sectional area of the samples in the study by Fithian et al. (6) (approximately 0.4 by 1.0 mm) was smaller than that of the samples in the Tissakht and Ahmed study (from 1.75 to 3.0 mm by 0.8 to 2 mm) (21). Since the circumferential collagen fiber bundles are 0.05-0.4 mm in diameter and are contained in a hydrophilic matrix (11), samples that can be prepared and subjected to a tensile test may contain differing concentrations of the hydrophilic matrix depending on their cross-sectional area. To reconcile this inconsistency in circumferential tensile modulus values, the following hypothesis was tested: the thickness (cross-sectional area) of the sample significantly affects the tensile modulus. If this hypothesis is accepted, then the cross-sectional area of the sample must be considered to determine a functional circumferential tensile modulus for the tissue.

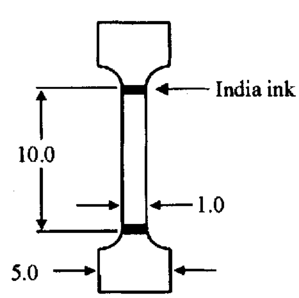

FIG. 2. The dimensional measurements (mm) of the custom precision punch. Waterproof India ink was applied at each end of the narrow portion of the sample for gauge length measurements (~10 mm). Gauge lengths varied due to small differences in the location and thickness of the India ink application.

MATERIALS AND METHODS

Experiments

Thirty human medial menisci (i.e., specimens), from cadavera 26-73 years of age, were obtained within 72 hours *post mortem* from regional tissue banks. Samples for testing were cut from each specimen in a manner similar to that described in previous studies (6,18). The peripheral surface of the meniscus was flattened against the freezing stage of a sledge microtome (Hacker Instruments, Fairfield, NJ, U.S.A.) by gently applying pressure to the anterior and posterior horns of the meniscus until the tissue was frozen in position. For each meniscus, the microtome was used to obtain a series of contiguous 0.5, 1.5, or 3.0-mm-thick slices. Three groups of 10 menisci each were prepared with one slice thickness. Slicing began on the inner edge of the meniscus with the blade oriented parallel to the peripheral rim (Fig. 1). The inner 2-3-mm slice of each meniscus was discarded because it was too narrow to test. A total of 6 mm of tissue was collected from each meniscus (i.e., 12 0.5-mm-thick slices, four 1.5-mm-thick slices, or two 3.0-mm-thick slices). Each slice was divided equally into anterior, central, and posterior regions. From each region, a custom punch was used to cut one dumbbell-shaped sample. The punch was oriented so that the long axis of the sample was parallel to the long axis of the slice. As was evident visually, this orientation provided a sample whose circumferential collagen fibers ran along the length of the sample. Samples were taken from the center of the slices. The narrow section of the sample was 1.0 mm wide and was defined as the gauge width (Fig. 2). With use of a digital micrometer (Mitutoyo, Minato-ku, Tokyo, Japan) with an accuracy of ±0.001 mm, the width and thickness were measured at three locations along the narrow section of the sample to calculate the cross-sectional area. Waterproof India ink was applied to the narrow portion of the sample to establish a gauge length of approximately 10 mm (Fig. 2). Squares of 600-grade polishing paper were glued to both ends of each sample with cyanoacrylate to provide a nonslip grip surface.

Tensile testing was performed with a servohydraulic materials testing system (model 858; MTS, Minneapolis, MN, U.S.A.) with a 98-N load cell (model SMT1-22; Interface, Scottsdale, AZ, U.S.A.). The materials testing system could resolve ±0.005 N. The bottom grip was rigidly attached within a bathing chamber to the base of the machine. To minimize tissue desiccation, the chamber was filled with saline solution at room temperature. The other grip was fixed to the load cell mounted to the vertical actuator. The sample was mounted in the grips with the circumferential collagen fibers oriented in line with the motion axis of the actuator. The sample was preconditioned to 3% strain at a displacement rate of 0.01 mm × s^{-1} for 10 cycles to provide a common strain history.

TABLE 1. *Mean (SD) circumferential tensile modulus (MPa) for samples according to circumferential location and sample thickness*

Thickness	Anterior	Central	Posterior
0.5 mm	141.2 (56.7) (s = 52)	116.4 (47.5) (s = 69)	108.4 (42.9) (s = 64)
1.5 mm	104.6 (63.8) (s = 23)	93.9 (49.1) (s = 25)	60.7 (40.6) (s = 28)
3.0 mm	72.0 (92.2) (s = 13)	43.4 (26.8) (s = 13)	67.1 (75.7) (s = 10)

To avoid pseudo-replication of the subject-to-subject variability, the tabulated values were calculated by averaging the data by specimen (i.e., an average of all anterior samples from one meniscus) and then averaging the resulting moduli from the menisci tested at each sample thickness to determine the mean and SD at a given circumferential location. Thus, n = 10 for most tabulated values since 10 menisci were tested for each slice thickness. However, the number of modulus values from samples tested successfully and used to calculate the averages for each region/thickness combination for all 10 menisci was generally greater than 10. The second number in parentheses is the total number of test samples that were used to calculate the average moduli for the 10 menisci for a particular region/thickness combination.

Under a tare load of 0.05 N, the initial gauge length (range: 6.5-10 mm) was recorded with a charge-coupled device video camera with a 50-mm lens and an image sensor containing 768 × 494 elements (model 4910; Cohu, San Diego, CA, U.S.A.). Images from the video camera were captured in a personal computer using a framegrabber card (model LG-3; Scion, Frederick, MD, U.S.A.). The sample was then filmed at 0.5 Hz while being pulled to failure at a rate of 0.006 mm × s^{-1}. This rate was chosen on the basis of a preliminary experiment within this laboratory and the results of previous studies to ensure quasi-static test conditions (17,21). The acquisition of the load and video elongation data was synchronized with use of an internal trigger to activate the camera. Samples that failed on or outside of the gauge length markers were discarded.

Data Analysis

The engineering stress and strain were calculated, and a stress-strain curve was constructed for each sample. Since the character of the curve was hardening nonlinear, the tensile modulus was calculated with use of simple linear regression of the stress-strain curve between 25 and 75% of maximum stress (Fig. 3).

Statistical analyses were performed with SAS software (Cary, NC, U.S.A.). To test whether the circumferential tensile modulus varied between the circumferential and radial locations within a meniscus, a mixed model analysis of variance (ANOVA) was performed in which the factors (circumferential and radial locations) were treated as random effects for each slice thickness. Since the specimen interactions were not significant, the model was reduced to ANOVA models that were blocked by specimen. These analyses were performed for each slice thickness. The independent variables were the two locations (circumferential and radial), and the dependent variable was the circumferential tensile modulus. The circumferential location had three levels (anterior, central, and posterior), and the number of levels for the radial location was dependent on the slice thickness (12 levels for the 0.5-mm slice thickness, four levels for the 1.5-mm slice thickness, and two levels for the 3.0-mm slice thickness). The gathered data were treated as if samples that could not be tested were excluded at random (i.e., a sample that was discarded was not related to the modulus that might have been measured at that location).

To test the hypothesis that the representative value for the circumferential tensile modulus was dependent on the slice thickness, a two-tailed *t* test for samples with unequal variance was used. A representative circumferential tensile modulus was calculated as the average modulus from all samples within each group of 10 menisci. The three representative circumferential tensile moduli were then compared. The Bonferroni adjustment was used to limit the experiment-wise Type-I error to 0.05 for the three paired comparisons ($p < 0.05/3$ for significance).

RESULTS

The circumferential tensile modulus did not vary significantly between the circumferential ($p = 0.900$,

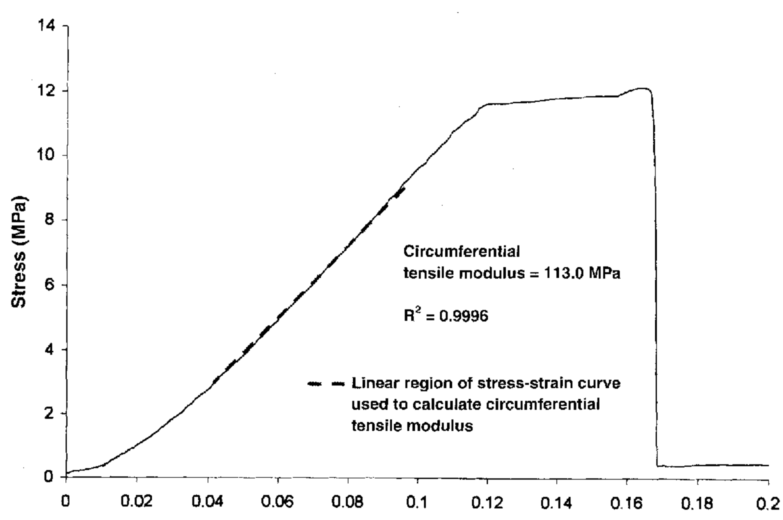

FIG. 3. Typical stress-strain curve. The dashed line represents the region of the curve used to calculate the circumferential modulus (R^2 = 0.9996).

TABLE 2. *Mean (SD) circumferential tensile modulus (MPa) for samples according to radial location and sample thickness*

Thickness	Inner	Outer
0.5 mm	120.3 (27.4) (s = 93)	116.9 (58.2) (s = 92)
1.5 mm	93.8 (25.2) (s = 39)	73.5 (49.9) (s = 37)
3.0 mm	42.9 (25.6) (s = 17)	52.5 (44.5) (s = 9)

The tabulated values were calculated by averaging the data by specimen for the inner 3 mm and the outer 3 mm of tissue and then averaging data from all of the specimens. Thus, n = 10 for most tabulated values since 10 menisci were tested for each slice thickness. However, the number of modulus values from samples tested successfully and used to calculate the averages for each region/thickness combination for all 10 menisci was generally greater than 10. The second number in parentheses is the total number of test samples that were used to calculate the average moduli for all 10 menisci for a particular region/thickness combination.

0.088, and 0.395 for 3.0, 1.5, and 0.5-mm thickness, respectively) and radial (p = 0.622, 0.371, and 0.991 for 3.0, 1.5, and 0.5-mm thickness, respectively) locations. Although the circumferential location of the sample had no significant effect on the circumferential tensile modulus, the average modulus for the anterior region was greater than that for the central or posterior region (Table 1). This trend was consistent for all three slice thicknesses. In contrast, no trend was evident between the radial location of the samples and the circumferential tensile modulus (Table 2).

The lack of any significant effect for either the circumferential or radial location on the circumferential tensile modulus justified the calculation of a representative or overall circumferential tensile modulus to evaluate the effect of slice thickness. After the Bonferroni adjustment, the *t* test results indicated that the overall circumferential tensile modulus at each sample thickness was significantly different from the others (Fig. 4). The average overall circumferential modulus was inversely proportional to the sample thickness (Fig. 4). The average modulus was 119.8 MPa for the 0.5-mm slice thickness, which was more than twice the average modulus of 49.8 MPa for the 3.0-mm slice thickness.

DISCUSSION

This study was conducted to determine the variability of the circumferential tensile modulus within the human meniscus and to determine if the slice thickness of the sample affected the measured modulus. To fulfill the study goals, an experimental approach was taken in which circumferentially oriented samples were collected from both circumferential and radial locations for three different slice thicknesses and were uniaxially pulled to failure.

Although the results indicated that the circumferential location of the samples did not affect the circumferential tensile modulus, the anterior region had a greater modulus than did either the central or posterior region for the same slice thickness. The meniscal ultrastructure is characterized by circumferential collagen fibers that run continuously from the anterior horn to the posterior horn (11); however, the anterior region of the medial meniscus is not as wide in the radial direction as is that of either the central or posterior region (22). Therefore, this region may have a slightly greater circumferential tensile modulus because the same numbers of collagen fibers are packed into a smaller cross-sectional area of tissue.

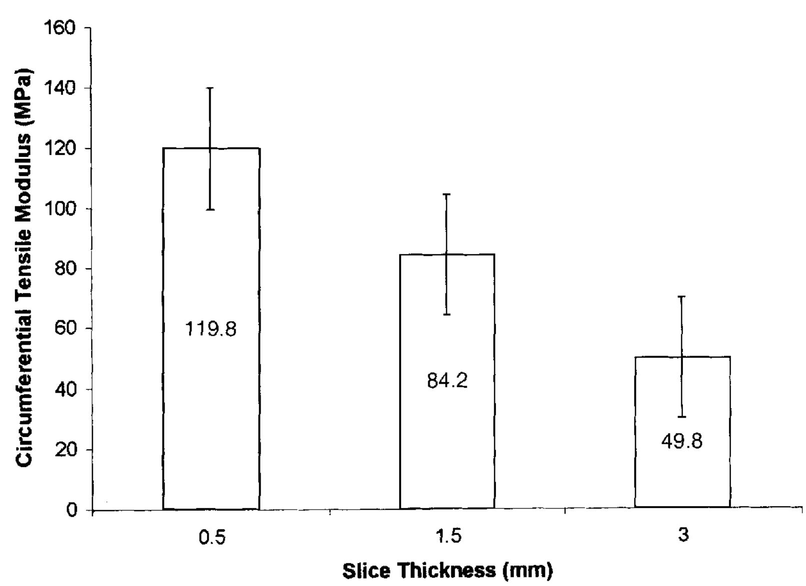

FIG. 4. Average overall mean (SD) circumferential tensile modulus (MPa) for all samples for the three slice thicknesses. The circumferential tensile modulus was inversely related to slice thickness. The modulus for the 0.5-mm-thick slice was significantly greater than that for the 1.5-mm (p = 0.004) or the 3.0-mm slice (p < 0.013). With the Bonferroni adjustment, p < 0.0167 indicates significance.

TABLE 3. *Comparison of the circumferential tensile modulus (MPa) as measured in the present study with previous results*

	Thickness × width of cross section (mm)	Modulus in anterior region (MPa)	Modulus in central region (MPa)	Modulus in posterior region (MPa)
Fithian et al. (6)	0.4 × 1.0	159.6	93.2	110.2
Tissakht and Ahmed (21)	1.75-3.0 × 0.8-2.0	91.2	76.8	81.1
Present study				
0.5 mm	0.5 × 1.0	141.2	116.4	108.4
1.5 mm	1.5 × 1.0	104.6	93.9	60.7
3.0 mm	3.0 × 1.0	72.0	43.4	67.1

Similar to the result for the circumferential location, the radial location of the sample did not significantly affect the circumferential tensile modulus. Initially, this finding appeared counterintuitive because the circumferential collagen fibers are more densely packed within the peripheral two-thirds of the meniscus than the inner one-third (1,3,7,15); therefore, peripheral samples would be expected to have a higher modulus than those from the inner third. However, the inner 2-3 mm portion of the meniscus was discarded during preparation of the slices because the tissue was too narrow to test. Since the radial width of the medial meniscus is 12 ± 3 mm (9), the discarding of 2-3 mm of the inner meniscus prevented most of the inner apex from being tested. Therefore, primarily the peripheral two-thirds of the meniscus was tested in this study.

Because there was no significant variation in the circumferential tensile modulus at different circumferential and radial locations, this study indicates that a representative (i.e., overall) circumferential tensile modulus can be calculated for the human medial meniscus by averaging the moduli measured at all locations. Although the entire meniscus was not represented in the data, a representative circumferential tensile modulus can be determined because the removal of the inner 2-3 mm resulted in an average of less than 7% of the meniscal tissue being discarded during preparation. Moreover, the inner one-third of the meniscus, which was discarded, is composed of irregularly woven collagen fibers (1,3,7,15) that would not be expected to contribute substantially to the overall circumferential tensile stiffness. Thus, the results reported in this study represent the material properties of the preponderance of the medial meniscus. The calculation of a representative circumferential tensile modulus would be useful both in modeling the meniscus within the knee joint and in future attempts to develop and select replacements for damaged menisci.

To properly interpret the results of this study, it must be recognized that the measurement of the tensile modulus was affected by the strain rate applied during testing because the meniscus is a viscoelastic material. The meniscus is composed of about 70% water (2,11) bound within a collagen matrix. The water is capable of flowing through the solid collagen matrix and out of the tissue as the meniscus is compressed (13,14). The rate of water efflux is controlled by the drag force, which is caused by this flow. Thus, the intrinsic, or flow-independent, material properties of the meniscus can be isolated by measuring the tensile modulus during tensile testing at a slow, constant strain rate. The dynamic, or flow-dependent, properties must include the frictional drag of the fluid flow through the solid matrix. The slow deformation rate used in the testing ensured that the frictional drag did not contribute to the modulus measured herein. Therefore, the circumferential modulus represents the biomechanical behavior of only the solid phase of the material.

The samples obtained from the thinnest slices (0.5 mm) were similar in cross-sectional area to those tested by Fithian et al. (6). Accordingly, the measured circumferential tensile modulus of 141.2 MPa reported in this study was similar to the modulus of 159.6 MPa reported by Fithian et al. (Table 3). Likewise, the middle-sized (1.5 mm) samples were within the range of the samples tested by Tissakht and Ahmed (21) and the circumferential tensile moduli were similar as well (104.6 and 91.2 MPa, respectively).

To ensure the validity of the statistical findings (no significant location effects), the powers of the statistical tests (two-factor ANOVAs) were calculated to determine the probability of a Type-II error (accepting the null hypothesis when it is not true) (16). Because larger numbers of test samples were collected from each meniscus for the thinner slices (185 samples for 0.5 mm, 76 samples for 1.5 mm, and 26 samples for 3.0 mm), the power was higher for the 0.5 mm (power = 0.995) compared with the 1.5 mm (power = 0.87) and the 3.0 mm (power = 0.51) slice thickness. This indicates that the probability of a Type-II error was low for the thinner samples and therefore that the null hypothesis (no significant variation throughout the tissue) can be accepted with confidence. Although the power for the thickest 3.0-mm slices was much lower, the conclusion from the test still appears valid. If there was any significant variation of the circumferential tensile modulus throughout the meniscus, then it should have been demonstrated within the thinner samples as well.

The inability to include the results from all samples may have contributed to the finding that the circumferential tensile modulus varied inversely with slice thickness. One reason that the results for all samples were not included was that some samples failed on or outside of the gauge lines (~20% for all slice thicknesses). Another reason was that other samples could not be tested due to failure during preparation or complete inability to obtain a sample at a given location. These samples could not withstand even the minimal stresses during preparation and either disintegrated or fell apart. The proportion of samples that could not be tested was highest for the thinnest slice thickness (28%) and was lower for the thicker of the two slices (17 and 18%). As a result, the circumferential tensile modulus for the thinnest slice thickness became artificially elevated because the samples with lower moduli were unable to be tested and hence were not included in the average. The requirements of obtaining a sample and performing a practical tensile test led to the finding that the modulus depends on the thickness of the test sample.

A theory for the higher failure rate for the thinnest slices can be offered based on the composition of the meniscal material. For a homogenous material, samples of any slice thickness would be expected to exhibit the same tensile modulus. However, since the meniscus is an inhomogeneous material composed of strong collagen fibers embedded within a weak hydrophilic matrix (1,3-5,8,12), the thickness of the sample slice may contribute to the measured circumferential tensile modulus. Some of the thinner samples may consist predominantly of the hydrophilic matrix and be largely devoid of collagen fibers. This was observed for the thinnest samples, which could not be tested. The thicker samples may be able to retain more of the hydrophilic gel within the sample, giving rise to the lower measured modulus.

Figure 5 offers an illustration of this point. Three thin slices through the area would produce two samples with approximately the same circumferential tensile modulus, whereas the middle sample would not contain any collagen fibers and would fail at a very low stress or prior to testing. However, if the same area was collected as one thicker sample, the sample area would increase by three but the number of collagen fiber bundles would increase only by two. Therefore, the stress for a given strain would decrease by 2/3 (force/area). A decrease in the stress would lead to a decrease in the circumferential tensile modulus when averaged over all radial locations and specimens.

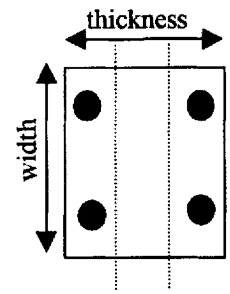

FIG. 5. Simplified cross-sectional representation of an area of meniscal tissue. The circles represent collagen fiber bundles. The dashed lines represent slicing of the the area into three thinner samples.

By the same example given previously for the slice thickness, the sample width may have affected the measured value of the circumferential tensile modulus. The sample width was selected to ensure that samples could be collected near the inner edge. Although a narrower sample width facilitated sample collection closer to the inner edge, this width could have led to a decrease in the measured circumferential tensile modulus. Accordingly, the overall modulus value obtained for the 3.0-mm slice thickness may still underestimate the functional (i.e., macroscopic) modulus of the tissue.

One source of error in calculating the modulus was the consistency of the cross-sectional area between slices and samples. Although both the microtome and punch are precise tools, slices were occasionally thinner after thawing (due to the expansion of the water freezing in the tissue) and the widths of the samples created by the punch were slightly different depending on the consistency of the meniscus in the region where the sample was obtained. To correct for these variations, the sample width and the slice thickness were measured with a digital micrometer. Use of this instrument also introduced error into the measurements because the tissue was soft and some judgment was necessary to determine contact. To quantify the nature of this error, a preliminary study compared measurements taken with the charge-coupled device camera with those taken with the micrometer. Unlike the effects of thawing, which introduced a systematic error, the error introduced by the micrometer was random in nature and hence did not affect the conclusions of the study because this type of error was managed by the statistical analysis.

In summary, although the results of this study support the concept that the meniscus is a complex, inhomogeneous material, they, along with those of Tissakht and Ahmed (21), indicate that the circumferential tensile modulus of the human meniscus does not show significant variation throughout the peripheral two-thirds of the meniscus. Thus, an overall circumferential tensile modulus can be calculated for a human meniscus. The circumferential tensile modulus may be lower than previously reported because prior samples may have been too small to go through a practical tensile test. An optimum sample size has not been determined; however, because larger samples can be tested with greater success than smaller samples, the results from testing larger samples may more accurately represent the material behavior of the tissue *in vivo*. Also, since the circumferential tensile modulus is inversely dependent on the slice thickness of the sample, the cross-sectional area of the sample should be considered when selecting an average circumferential tensile modulus for finite element modeling. These findings should contribute to future studies of the biomechanical behavior of the menisci using mathematical and *in vivo* models to develop meniscal replacements that duplicate the behavior of the intact meniscus.

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