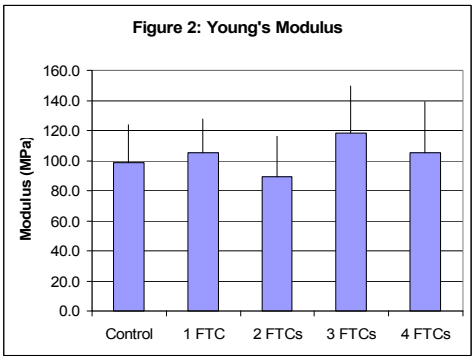
The Effects of Freezing on the Circumferential Tensile Properties of Meniscus

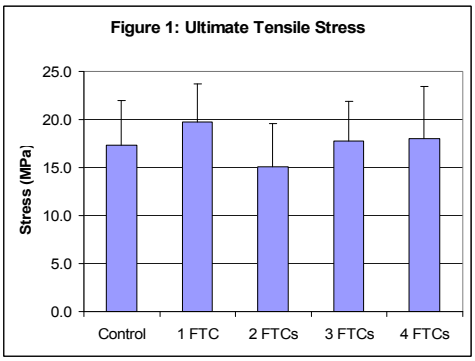
C. P. Skurla[1], D. S. Morgan[1], C. T. Towe[2]
[1]Baylor Univ., Waco, TX, [2]St Louis Children's Hosp., St Louis, MO

Statement of Purpose: Numerous studies that characterized the circumferential tensile properties of meniscus have been published; however, the effects of repeated freeze-thaw cycles (FTCs) on the mechanical properties of meniscus have not been thoroughly characterized. For example, in one study, fresh canine menisci (i.e., controls) and cryopreserved menisci were tensile tested and properties were compared. The conclusion of the study was that freezing did not affect the tensile properties; however, the control specimens underwent 2 FTCs during sample preparation prior to testing[1]. The objective of the present study was to determine the effect of FTCs on the circumferential tensile properties of bovine meniscus. The null hypothesis of the study was that multiple FTCs would not significantly affect the mechanical properties of the specimens.

Methods: One freeze-thaw cycle (FTC) was defined as storing the specimen for five days in a freezer at -20°C followed by thawing for two days in a refrigerator at 1.4°C. Five treatment groups were chosen: Control (i.e., fresh), 1 FTC, 2 FTCs, 3 FTCs, and 4 FTCs. A power calculation determined the sample size per group to be twelve. Fresh bovine knees were obtained at a local abattoir, and the medial menisci were harvested. The menisci were visually inspected, and specimens that appeared undamaged and pearly white were selected for the study. One specimen was defined as one meniscus, due to large variability in mechanical properties among bovine menisci, as determined in a previous study[2-3]. Specimens were wrapped in isotonic saline-soaked gauze sponges and placed in plastic freezer bags for storage. The control group was mechanically tested on the date of harvest. All other specimens were treated for the appropriate number of FTCs. A wedge-shaped section was cut from the center of the meniscus. The block wedge was trimmed and glued with cyanoacrylate glue to a hand microtome. A custom-built cutting device was used to cut slices, parallel to the tibial plane, approx. 200 μm thick. Five to 15 slices were cut from each meniscus and tested. The slice was trimmed to yield a test specimen, approx 15 mm x 7.5 mm, from the middle third (i.e., avascular zone) of the slice. Thickness of the slice was measured using a dial caliper. Sandpaper strips were glued, using cyanoacrylate glue, to either end of the test specimen to provide a better surface for gripping in the test jigs. All slices were kept moist with isotonic saline during specimen preparation and testing. Mechanical testing was performed using an MTS Mini Bionix II system (MTS Corp, Eden Prairie, MN) at a distraction rate of 0.5 mm/min until a 1N pre-load was reached, at which time the specimen was photographed for later digital analysis. Testing continued at the same distraction rate until failure. After failure, specimens were visually inspected to verify that they failed within the gage length and not at the glue joints. Specimens that failed at the glue joint were eliminated. Digital image analysis (Scion Image Beta 4.0.2, Scion Corp, Frederick, MD) was performed to determine specimen length and width. Data analysis was performed using SAS (SAS Institute, Cary, NC). Ultimate tensile strength and modulus of elasticity were determined for each test specimen. Values for each individual bovine meniscus were averaged, and the averages were used in subsequent data analysis. Analysis of Variance (ANOVA) was used to determine if any groups were significantly different from each other, followed by Tukey-Kramer multiple comparisons.

Results: There were no significant differences ($p<0.05$) among groups for either ultimate tensile stress or Young's modulus (See Figures 1 & 2 below). The null hypothesis was not rejected. A qualitative observation was made during slicing of the menisci. Fresh menisci felt soft and rubbery, but as the number of FTCs increased the menisci became increasingly stiff and hard and increasingly easy to slice.





Conclusions: The circumferential tensile properties of bovine menisci, which can be attributed to the tensile strength of the circumferentially-oriented collagen fibers, do not appear to change with increasing numbers of FTCs. However, observations regarding the texture of the menisci indicate changes in viscoelastic properties. Future work includes mechanical testing in confined compression[4] to compare the viscoelastic properties among FTC treatment groups.

References: [1] Arnoczky SP. J Orth Res. 1988; 6:1-12. [2] Towe CT. Trans HSEMB. 2004; 21. [3] Skurla CP. J Biomech. 2007; 40: 220-224. [4] Mow VC. Ch 4 in Basic Orth Biomech, 2nd ed. 1997. Lippincott-Raven.