

NIH Public Access

Author Manuscript

J Biomech. Author manuscript; available in PMC 2011 February 10.

Published in final edited form as:

J Biomech. 2010 February 10; 43(3): 463. doi:10.1016/j.jbiomech.2009.09.043.

GEOMETRY, TIME DEPENDENT AND FAILURE PROPERTIES OF HUMAN MENISCAL ATTACHMENTS

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Abstract

Meniscectomies have been shown to lead to osteoarthritis and the success of meniscal replacements remains questionable. It has been suggested that the success of a meniscal replacement is dependent on several factors, one of which is the secure fixation and firm attachment of the replacement to the tibial plateau at the horn locations. To aid in the development of meniscal replacements, the objectives of the current study were to determine the time-dependent and failure properties of human meniscal attachments. In contrast to the time-dependent tests, during uniaxial failure testing a charge-coupled video camera was used to document the local strain and linear modulus distribution across the surface of the attachments. The lateral attachments were statistically smaller in cross-sectional area and longer than the medial attachments. The anterior attachments were statistically longer and had a smaller cross-sectional area than the posterior attachments. From the stress relaxation tests, the load and stress relaxation rates of the medial anterior attachment were statistically greater than the medial posterior attachment. There were no significant differences in the creep, structural properties or the ultimate stress between the different attachments. Ultimate strain varied between attachments as well as along the length of the attachment. Ultimate strain in the meniscus region $(10.4\pm6.9\%)$ and midsubstance region $(12.7\pm16.4\%)$ was smaller than the bony insertion region $(32.2\pm21.5\%)$. The lateral and anterior attachments were also found to have statistically greater strain than the medial and posterior attachments, respectively. The linear modulus was statistically weaker in the bony insertion region (69.7 \pm 33.7 MPa) compared to the meniscus region (153 \pm 123 MPa) and mid-substance region $(195\pm121 \text{ MPa})$. Overall the anterior attachments $(169\pm130 \text{ MPa})$ were also found to be statistically stronger than the posterior attachments (90.8 ± 64.9 MPa). These results can be used to help design tissue engineered replacement menisci and their insertions and show the differences in material properties between attachment as well as within an attachment.

Keywords

meniscus; knee; horn attachments; enthesis; modulus; material properties

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Conflict of Interest Statement:

The authors affirm that they have no financial affiliation or involvement with any commercial organization that has direct financial interest in any matter included in this manuscript.

INTRODUCTION

Menisci serve several important functions in the knee joint including load bearing and transmission across the joint (Walker and Erkman, 1975; Shrive et al., 1978; Radin et al., 1984). The geometry, composition, and firm attachment of the menisci to the tibial plateau allow it to bear and distribute load across the knee joint (Fithian et al., 1990; Renstrom and Johnson, 1990; Setton et al., 1999). Menisci are frequently injured and often treated by partial or total meniscectomy if the injury occurs in the avascular zone. Meniscectomies have been shown to lead to decreased contact areas, increased stress, and degeneration of articular cartilage (Baratz et al., 1986; Chen et al., 1996; Szomor et al., 2000; Zielinska and Haut Donahue, 2006). Therefore, meniscal replacements are under investigation. Effective meniscal replacements aim to restore the native contact mechanics of the knee. Factors affecting the function of meniscal replacements include: the method of fixation to the tibial plateau (Chen et al., 1996; Alhalki et al., 1999; Setton et al., 1999; Cole et al., 2003), the size, geometry and material properties of the replacement (Pollard, 1995; Setton et al., 1999).

Meniscal attachments are thought to be ligamentous and previous studies have characterized the time dependent (Maes and Haut Donahue, 2006) and failure properties of bovine meniscal attachments (Villegas et al., 2007). Although these studies have provided more insight on meniscal attachments, one pitfall is that in the bovine knee the lateral posterior enthesis attaches to the femur, unlike a tibial insertion in the human. The current study will expand the knowledge of the mechanical response of human meniscal horn attachments in order to aid in the development of more successful meniscal replacements. The objectives of the present study were to 1) examine the stress relaxation and creep properties of human meniscal attachments, 2) determine the failure properties of human meniscal attachments, and 3) examine the local strain and linear modulus distribution across the surface of the attachments.

MATERIALS AND METHODS

Specimen preparation

Six human knees (51-67, avg. age 59 yrs; 5 males, 1 female) were obtained from a national tissue bank and frozen (NDRI, Philadelphia, PA). The sample size used in this study was not large enough to study differences due to age or gender. Prior to testing, each knee was thawed at room temperature and dissected. A non-destructive method of accurately measuring the cross-sectional area of irregular shapes was used to measure the area (Race and Amis, 1996; Goodship and Birch, 2005). The length measurements were taken parallel to the collagen fibers at three locations; outer, middle, and inner (Figure 1) and on both the proximal and distal sides of each attachment.

The tibia was potted in a steel tube and mounted in a custom built 5 DOF fixture previously used (Maes and Haut Donahue, 2006). Meniscal attachments were loaded parallel to the collagen fiber orientation and tibial plateau while bathed in 37°C PBS using a servo-hydraulic uniaxial materials testing machine (Model 8872, Instron Corporation, Canton, MA) (Figure 2). To ensure uniaxial tension and reduce slippage, a universal joint was used with a cryoclamp. Care was taken to ensure that the zone of frozen tissue did not extend beyond the freeze clamp and the freeze line did not penetrate into the tissue being tested.

The meniscus was gripped at the transition line between the meniscal tissue and attachment while the tibia was held fixed. Each attachment was preconditioned for 10 cycles at 10mm/ min, between 0% and 3% of the gauge length using a sine wave (Maes and Haut Donahue, 2006). Each attachment of a given specimen was first tested in stress relaxation, followed by a recovery period (Maes and Haut Donahue, 2006) prior to a creep test. Lastly, following the creep test and a recovery period, each attachment was pulled to failure.

The stress relaxation test ramped to a deformation of 3% of the gauge length in 0.5 seconds and then held the deformation for 45 minutes (Hingorani et al., 2004). All four attachments were tested in random order from each specimen. The specimen was then placed at 4 C and allowed to fully recover (Maes and Haut Donahue, 2006). The creep test ramped to the peak load determined from the stress relaxation test (Hingorani et al., 2004) in 0.5 seconds and was held for 45 minutes. Again, attachments were tested in random order and allowed to recover. The creep and stress relaxation rates were determined by plotting against the natural log of time and finding the slope using linear regression. One sample slipped from the grip during time-dependent testing, hence, that knee data was not included in the data analysis and results.

In order to increase the sample size for failure testing data, we tested an additional 2 knees with an average age of 55, 1 female and 1 male. Testing of the additional knees was completed to ensure at least 5 samples of each attachment were tested. Not all sample sizes were equal to 8 however since some samples slipped, errors with the data acquisition system occurred and some attachments failed at the grip interface. The pull to failure test ramped at a rate of 2% sec⁻¹ (Lam et al., 1995; Quapp and Weiss, 1998). A 3×3 grid was created on the tissue surface, dividing the attachment into three horizontal regions and three longitudinal sections (Figure 3). The Meniscus (ME) region was defined as the upper region near the meniscus, the Midsubstance (MI) was defined as the middle region of the attachment, and the Bony Insertion (BO) was defined as the lower region near the insertion into the bone. Longitudinally, the attachment was divided into Outer, Middle, and Inner sections (Figure 3). A charge-couple video camera (Model MicroPix M-1024 CCD camera, Ann Arbor, MI) recorded motion of the markers. Pictures captured from the camera were analyzed using a custom-made processing program (MATLAB, version 7.4 (R2007a)) in order to calculate Green's strain (Villegas et al., 2007). Both structural and material properties were determined from the failure tests. To quantify the structural properties of the attachments the ultimate load (N), the ultimate elongation (mm), and the linear stiffness (N/mm) were determined. The linear stiffness was defined as the slope of the linear region of the load versus displacement plot and was determined using linear regression. To quantify the material properties of the attachments the ultimate stress (MPa), the ultimate strain (%), and the linear modulus (MPa) were determined. The linear modulus was defined as the slope of the linear region of the stress versus strain plot using linear regression.

Averages and standard deviations were calculated for dimensional properties, stress relaxation and creep properties, structural properties, and ultimate stress for each of the four attachments and a oneway repeated measures analysis of variance (ANOVA) was performed to make comparisons between attachments. The descriptive statistics were divided into longitudinal sections for each region for each attachment as well as longitudinal sections for each region for each side (lateral, medial) and each location (anterior, posterior). A four factor (side, location, horizontal region, longitudinal section) ANOVA was performed to compare the ultimate strains and linear moduli between the side and location of the attachments and between the horizontal regions and longitudinal sections. When significant results were identified by ANOVA, post hoc comparisons were made using Tukey's method ($p<0.05$). Comparisons were also made between the combination of side and location to test differences in attachments. A significance level of 0.05 was used for all statistical analyses.

RESULTS

The proximal surface $(11.9\pm3.97 \text{ mm})$ was statistically longer than the distal surface (10.3 m) ± 3.93 mm). The outer side of the attachment (13.1 ± 4.61 mm) was statistically longer than the inner (9.08 \pm 2.93 mm) and middle (11.5 \pm 3.29 mm). The middle length was also found to be statistically longer than the inner length. Although significant differences were found between

The MP attachment was statistically shorter than all other attachments and the LP attachment was statistically shorter than the MA and the LA attachments (Table 1). The lateral attachments were 5% longer than the medial attachments ($p=0.02$), and the anterior attachments were 36% longer than the posterior attachments. The lateral attachments were 6% smaller in crosssectional area than the medial attachments, and the cross-sectional area of the anterior attachments were 20% smaller than the posterior attachments (Table 1).

The only significant differences in stress relaxation properties were found between the MA and MP attachments (Table 2). No significant statistical differences were found between any of the attachments for the creep properties $(p>0.05)$. The MA attachment showed the largest values for many creep properties, while the MP attachment showed the smallest values for these properties (Table 3).

While no significant differences between the ultimate load, ultimate elongation, linear stiffness or ultimate stress were found between the four attachments ($p > 0.05$) (Table 4), the ultimate loads and ultimate elongations tended to be larger for the LA and MP attachments compared to the LP and MA attachments. The linear stiffness had a 40% difference among the attachments with the LA attachment approximately double the LP attachment.

The mean ultimate strain of the lateral attachments $(20.1 \pm 20.6\%)$ was statistically greater than the medial attachments (17.7 \pm 16.5%), while the anterior attachments (20.2 \pm 20.5%) were statistically greater than the posterior attachments (17.6±16.9%). Ultimate strains were also statistically different between individual attachments with the MP $(12.8\pm8.50%)$ statistically smaller than both the LA (20.6±22.2%, p=0.03) and MA (19.7±18.6%, p=0.04) attachments. The ultimate strain of the BO region $(32.2\pm 21.5\%)$ was statistically over 50% greater than both the ME (10.4 \pm 6.9%, p=0.00) and MI regions (12.7 \pm 16.4%, p=0.00) (Table 5).

The mean linear modulus of the anterior attachments (169 ± 130 MPa) were statistically stronger than the posterior attachments (90.8 \pm 64.9 MPa, p=0.00) while no significant differences were found between the lateral and medial attachments. Linear modulus values among the attachments were statistically important, with the LA attachment $(161\pm124 \text{ MPa})$ and MA attachment (179 \pm 139 MPa) statistically stronger than the LP attachment (96.3 \pm 70.7 MPa, $p<0.00$ (Table 6). As presented above the ultimate strains were higher in the BO region, which corresponded to a decreased linear modulus at the bone insertion site. The ME region (153 ±123 MPa) and MI region (195±121 MPa) were statistically over 50% stronger than the BO region (69.7 \pm 33.7 MPa, p=0.00). Again no statistic differences were found between the outer, middle, and inner sections of the meniscal attachments $(p>0.05)$ (Table 6).

DISCUSSION

The research presented here documents for the first time, the geometry, time-dependent, and failure properties of human meniscal attachments. Relaxation of the attachments could affect the attachment response to repetitive loading, which may change the position of the meniscal replacement within the joint. The variations in material properties seen between different attachments and within an individual attachment may be related to different loading conditions in the knee or to biochemical differences. From these complex results it appears that meniscal replacements may need to possess properties that varying with location in the joint, as well as within the attachment to replicate the native attachments.

Specimens in this study underwent a free thaw cycle. While a previous study has shown no significant change in properties of soft tissues after freeze/thaw cycles (Clavert et al., 2001),

failure strength and Young's modulus have been found to be statistically smaller for freeze/ thaw specimens compared to fresh specimens (Sabiston et al., 1990; Clavert et al., 2001). Thus future studies should consider testing fresh material when possible. Additionally, only the surface strain was measured during the tests, and thus it is uncertain what the strain distribution is throughout the thickness of the attachment. Additionally, the strain measurements were taken from 2D images and no correction factor was used to account for any curvature of the meniscal root surface. With avulsion failures it is believed that the strains and loads seen at failure may not accurately represent the strain and strength of the actual ligament as it does the ligament bone interface (Woo et al., 1983). Considering this, care should be taken in interpreting these results as 50% of the samples failed by bone avulsion at the tibial plateau. Uniform loading of all fibers of the cross-section was difficult to confirm. However, since there were no significant difference in surface strains from the inner to middle to outer sections, it is likely that the crosssection was loaded evenly. Lastly, it is presently unknown if these property variations will be necessary for a meniscal replacement to restore normal knee kinematics and contact behavior. Future studies using finite element simulations can answer this question (Haut Donahue et al., 2003).

While the menisci are also attached via meniscofemoral ligaments (MFL) and the transverse ligament, the study of these attachments was beyond the scope of this study. Properties of the MFL have previously been documented (Gupte et al., 2002). The transverse ligament connects the anterior horns of the medial and lateral menisci. Although its material properties are still unknown, it would require a different experimental set-up to test 2 soft tissue interfaces

It has previously been shown that the anterior region of the human meniscus displaces more than the posterior region during motion (Thompson et al., 1991; Vedi et al., 1999; Rankin et al., 2006). The geometric results show that the anterior attachments are longer and smaller in cross-sectional area in comparison to the shorter and larger posterior attachments. Perhaps this loading environment has resulted in meniscal attachments that have adapted to fit in the joint space. The lateral meniscus has also been shown to translate more than the medial meniscus (Thompson et al., 1991; Vedi et al., 1999; Rankin et al., 2006) and in the same fashion the lateral attachments were found to be longer and smaller in cross-sectional area than the medial attachments. Although significant differences were found in the geometry of the meniscal attachments, no significant structural differences in ultimate elongation or stiffness were found.

The results of the stress relaxation tests demonstrated that the meniscal attachments are time dependent, with significant differences between the MA and MP attachments. In the stress relaxation study of bovine attachments (Maes and Haut Donahue, 2006), it is interesting to note that the normalized load and the stress at the end of the test are approximately half of that in the current human study and the normalized creep rate was 20-200 times larger for the bovine.

Due to the ligamentous nature of the attachments, their properties can be compared to other ligaments. From a number of studies linear stiffness values have ranged from 30-120 N/mm, ultimate loads from 290-1100 N, and ultimate stress from 13-70 MPa for various knee ligaments (Johnson et al., 1994; Harner et al., 1995; Staubli et al., 1999; Gupte et al., 2002; LaPrade et al., 2005). In the current study the average values were found to be 172 N/mm, 479 N, and 25.2 MPa for the linear stiffness, ultimate load, and ultimate stress, respectively, in general agreement with other ligamentous structures of the knee. These data should be interpreted with care as the variability is quite high and whether the variability is a result of a large natural variation or inherent to the methods used is not known. However, a similar setup has been used to test other soft tissues with success (Haut and Powlison, 1990; Haut and Haut, 1997; Donahue et al., 2001). While no significant differences were found between the ultimate load of the medial and lateral posterior attachments, the medial posterior attachments had a substantially larger ultimate load compared to the lateral posterior attachments. Clinically

it is often shown that the medial attachment is torn more often (Smith et al., 2002), and hence, a possible explanation would be that *in vivo* the meniscofemoral ligaments (MFL) contribute to the strength of the lateral posterior attachment. Ultimate strain, load and elongation were not reported if the attachment failed at the grip interface. Using sensitivity data from Haut Donahue et al., 2003 and the standard deviation from this study, the power of failure data was only 70% (Haut Donahue et al., 2003). Hence, the lack of statistically significant differences in the failure data should be interpreted with consideration of the power of the test.

The ultimate strain distribution across the surface of the attachment was found to be inhomogeneous. Similar to other studies of knee ligaments (Woo et al., 1983; Lam et al., 1995; Gardiner et al., 2001), the greatest strains in the current study were found nearest to the insertion site compared to that of the midsubstance region. Collagen fibril crimp frequency was found to be largest in the BO zone compared to the ME and MI zones of bovine meniscal attachments (Villegas et al., 2008). Higher crimp frequency allows for more stretching of the fibers, corresponding to greater strains in this region. Large strains at the insertion site correlates with reduced linear modulus in this region compared to the midsubstance, another phenomenon that was captured in the current study. Several studies have shown avulsion as a common mode of failure in knee structures (Woo et al., 1983; Lam et al., 1995; Gao et al., 1996) which corresponds to larger strains being found near the insertion sites of the ligaments to bone. The present study reaffirms this finding with 50% of the attachments failing by tibial avulsion.

The clinical implications of these results indicate that the design of meniscal replacements will be a challenging process in which independent detail will need to be given to each of the four attachments. If the same properties were applied to all four attachments for example the posterior attachments low mobility may be compromised changing joint mechanics. This knowledge can also help in the design of meniscal replacements used with knowledge of the different loading and motions of the meniscus in these different locations. Most importantly it was found that the strain and therefore linear modulus distribution along the length of the attachment surface are different. The attachments of meniscal replacements will likely need to replicate this variation in material properties along the length to replicate the gradient of strength from the meniscus body down into the underlying bone at the insertion site and prevent failure. Posterior root tears are becoming increasing evident (Brody et al., 2006; Jones et al., 2006; Choi et al., 2008; Lee et al., 2008; Ahn et al., 2009; De Smet et al., 2009), and hence, this data will not only have relevance in the design of meniscal replacement, but for the repair of root tears.

Acknowledgments

This study was supported in part by the National Institutes of Health (AR051906-01).

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Figure 1.

Superior view of the lateral anterior (LA) attachment, showing the transition from meniscus to attachment and the bone insertion site. Regions of length measurement are also labeled (outer – O, middle – M, inner- I).

Figure 2.

Schematic of test setup with fixture/bath assembly, clamp assembly, and loaded specimen.

MENISCUS

Table 1

Dimensional properties of human meniscal attachments (LA – lateral anterior, LP – lateral posterior, MA – medial anterior, MP – medial posterior). Average ± standard deviation

*** Significantly different from LP (p<0.05)

^ Significantly different from MA (p<0.05)

Significantly different from MP (p<0.05)

~ Significantly different from medial (p<0.05)

*@*Significantly different from posterior (p<0.05)

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Statistically different from MP (p<0.05) Statistically different from MP (p<0.05)

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Creep properties of human meniscal attachments (LA - lateral anterior, LP - lateral posterior, MA - medial anterior, MP - medial posterior). Average ± Creep properties of human meniscal attachments (LA – lateral anterior, LP – lateral posterior, MA – medial anterior, MP – medial posterior). Average ± standard deviation. Linear curve fits of the normalized data plotted against the log of time was >80%. standard deviation. Linear curve fits of the normalized data plotted against the log of time was >80%.

Table 4

Structural and material properties of human meniscal attachments obtained from pull to failure tests (LA – lateral anterior, LP – lateral posterior, MA – medial anterior, MP – medial posterior). Average ± standard deviation

No significant differences (p>0.05) n=5

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Ultimate strain data of human meniscal attachments obtained from pull to failure tests (LA - lateral anterior, LP - lateral posterior, MA - medial anterior, MP - medial posterior). Average ± standard deviations. No statistical differences were found between the outer, middle, and inner sections of the meniscal MP – medial posterior). Average ± standard deviations. No statistical differences were found between the outer, middle, and inner sections of the meniscal Ultimate strain data of human meniscal attachments obtained from pull to failure tests $(LA - later$ al anterior, LP – lateral posterior, MA – medial anterior, attachments (p>0.05) attachments (p>0.05) I

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*#*Significantly different from MP

 $^\#$ Significantly different from MP

*1*Significantly different from MEDIAL

*2*Significantly different from POSTERIOR

 $\boldsymbol{^{2}S}$ ignificantly different from POSTERIOR $I_{\mbox{Significantly}}$ different from MEDIAL

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Table 6

Linear modulus data of human meniscal attachments obtained from pull to failure tests (LA – lateral anterior, LP – lateral posterior, MA – medial anterior, MP – medial anterior, MP – medial anterior, Linear modulus data of human meniscal attachments obtained from pull to failure tests (LA – lateral anterior, LP – lateral posterior, MA – medial anterior, MP – medial posterior)

J Biomech. Author manuscript; available in PMC 2011 February 10.

Significantly different from BO *#*Significantly different from LP

 $^\#$ Significantly different from LP

*1*Significantly different from POSTERIOR

 $I_{\mbox{Significantly}}$ different from POSTERIOR