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Journal of Biomechanics 39 (2006) 3055–3061

JOURNAL
OF
BIOMECHANICS

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Time dependent properties of bovine meniscal attachments: Stress relaxation and creep

Jason A. Maes[1], T.L. Haut Donahue*[,2]

Department of Mechanical Engineering, Michigan Technological University, Houghton, MI 49931, USA

Accepted 15 September 2005

Abstract

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 [Chen, M.I., Branch, T.P., et al., 1996. Is it important to secure the horns during lateral meniscal transplantation? A cadaveric study. Arthroscopy 12(2), 174–181; Alhalki, M.M., et al., 1999. How three methods for fixing a medial meniscal autograft affect tibial contact mechanics. American Journal of Sports Medicine 27(3), 320–328; Haut Donahue, T.L., et al., 2003. How the stiffness of meniscal attachments and meniscal material properties affect tibio-femoral contact pressure computed using a validated finite element model of the human knee joint. Journal of Biomechanics 36(1), 19–34]. The complex loading environment in the knee lends itself to different loading environments for each meniscal attachment. We hypothesize that the creep and stress relaxation characteristics of the horn attachments will be different for the anterior versus posterior, and medial versus lateral attachments. To test this hypothesis, the stress relaxation and creep characteristics of the meniscal horn attachments were determined. The stress relaxation properties of load/stress at the end of the test, and the load/stress relaxation rate demonstrated no significant statistical differences between the attachments. Unlike the stress relaxation properties, the creep properties demonstrated some significant differences amongst the attachments. The normalized displacement at the end of the test, normalized creep rate and strain creep rate for the lateral anterior attachment were significantly different than those of the medial posterior attachment ($p < 0.05$). The two anterior attachments had significantly different strains at the end of the test, as well as significantly different creep strain rates ($p < 0.05$). The two attachments of the medial meniscus revealed no significant differences between any of the creep properties measured ($p > 0.05$). The time dependent properties obtained in this experiment provide insight into the behavior of meniscal horn attachments under various loading situations. The results indicate that a suitable meniscal replacement may require different properties for the lateral and medial horns.

Keywords: Meniscus; Time dependent; Stress relaxation; Creep; Horn attachments

1. Introduction

The menisci are fibrocartilagenous structures of the knee joint that aid in joint lubrication and stability. Meniscal tears result from traumatic injury as well as degeneration. When repair is not possible, such as tears in the avascular region, or complex tears, meniscal replacement is the next best solution (Sweigart and Athanasiou, 2001). The native menisci are primarily anchored to the tibial plateau through attachments at their anterior and posterior horn. The structure of meniscal horn attachments has been mainly considered as ligamentous in nature, with fibers oriented parallel to the direction of loading (Woo et al., 1997). In order to replace a damaged meniscus, a number of biomechanical criteria must be met, including geometry, material properties and attachment.

Previous experimental and theoretical studies suggest that attachment of a meniscal replacement can influence its ability to restore normal joint contact mechanics (Chen et al., 1996; Alhalki et al., 1999; Haut Donahue

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*Corresponding author. Tel.: +1 906 487 2078; fax: +1 906 487 2822.

E-mail address: thdonahu@mtu.edu (T.L. Haut Donahue).

[1] Research Assistant.

[2] Assistant Professor.

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doi:10.1016/j.jbiomech.2005.09.025

et al., 2003). Previous computational modeling work has shown that changes in the stiffness of horn attachments changed the knee joint contact pressure behavior by as much as 25% over the lateral and medial hemijoints (Haut Donahue et al., 2003). While it is evident that the meniscal attachments are important for proper function of the meniscus, little or no data has been reported on the properties of these attachments. Goertzen et al., 1996a, b reported the tensile failure loads of rabbit meniscal attachments, and found the medial anterior meniscal attachment to have a higher failure load than the medial posterior attachment. Meniscal attachments serve as the transition from meniscal fibrocartilage into the subchondral bone. Meniscal attachments, like ligaments, resist repetitive loading (Holden et al., 1994). Ligament replacement grafts have been shown to elongate up to 3 yr after implantation (Noyes, 1992). Quantifying the time dependent creep response of meniscal attachments is important, since the knee is subjected to repeated loading and this will, in most probability, cause the meniscal attachments to creep over time. Furthermore, for replacement menisci, the attachment may be placed under pretension upon implantation, which can relax over time. Hence, not only is the creep behavior important, but equally important is the stress relaxation behavior, since both of these are likely to contribute to the motion of the meniscal replacement upon joint loading. Currently, the authors are unaware of any previous work on the time dependent properties of meniscal attachments.

As the knee joint flexes from 0 to 90°, the medial anterior meniscal attachment displaces over two times more than the posterior attachment, and it is thought that the posterior attachment is not only in tension, but sees significant compressive loads as well (Thompson et al., 1991). Due to the differences in loading environment between the various attachments, we hypothesize that the creep and stress relaxation characteristics of the horn attachments will be different for the anterior versus posterior and medial versus lateral attachments. The objective of the proposed study is to enhance the current understanding of the biomechanical properties of the native meniscal attachments so as to design tissue engineered replacements.

2. Methods

2.1. Specimen preparation

Eight bovine stifle (knee) joints, with an age range between 15 and 30 months, were obtained from a slaughterhouse and frozen at −20 °C. On the day of testing, specimens were thawed at room temperature and disarticulated. All tissues were removed, leaving only the proximal tibia with the menisci and their attachments intact. The tibia of each specimen was potted using commercially available Fibre-Strand body filler 6371 (The Martin Senour Company, Cleveland, OH). Throughout the preparation, the attachments and menisci were kept moist with saline saturated gauze.

After the filler had set, the specimen was mounted into a custom fixture, which was submerged in a bath of 0.15 M NaCl saline solution at 37 °C (Sigma–Aldrich Co., St. Louis, MO). The fixture provides five degrees of freedom (Fig. 1), allowing the tibia to be rotated about three axes and translated along two axes, to establish physiological alignment of each specimen, such that the attachments were loaded parallel to their collagen fiber orientation and tibial plateau (Fig. 2), as they would be in situ. The fixture and bath were placed in a servo-hydraulic uniaxial materials testing machine (Model 8872, Instron Corp., Canton, MA). A custom-designed clamp was built to grasp the meniscal tissue (Riemersa and Schamhardt, 1982; Butler et al., 1984).

The cross-sectional area of the attachments was measured using an area micrometer (Ellis, 1969; Noyes and Grood, 1976; Butler et al., 1984). The lengths of the specimens were measured using digital calipers, from the tibial insertion to the transition between the attachment and the meniscal tissue, parallel to the collagen fiber orientation. The transition line between the attachment and the meniscus, the bone insertion site and the collagen fiber orientation were visually distinct

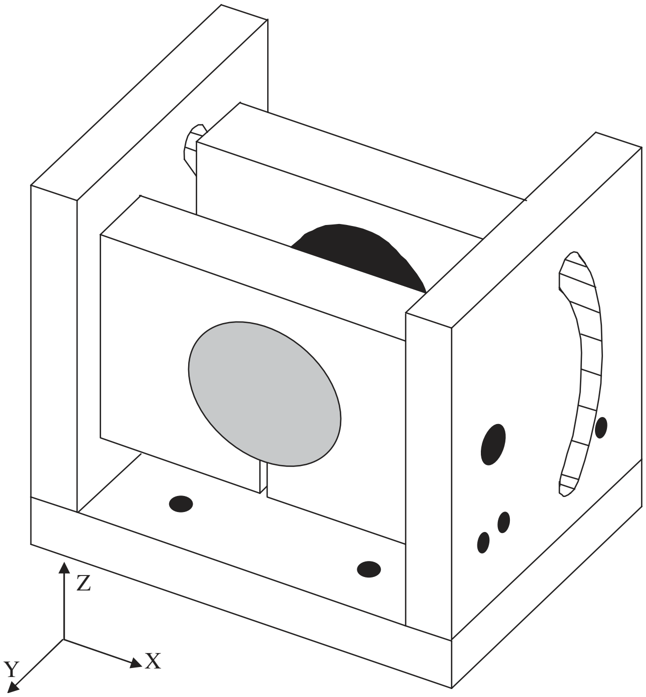

Fig. 1. Isometric view of test fixture used to provide five-degrees of freedom for physiological alignment of the attachments during testing. Fixture allows rotation about the x, y, and z axes, and translation in the x and y directions.

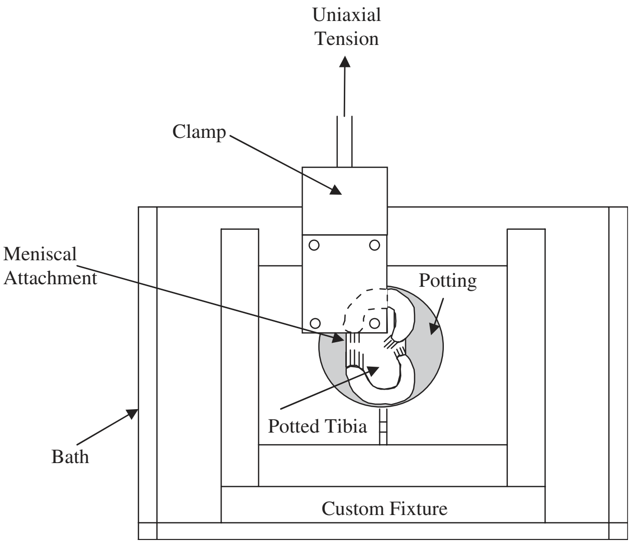

Fig. 2. Schematic of the test set-up. The test fixture and clamp allowed uniaxial tension to be applied to the meniscal attachment, replicating in situ conditions.

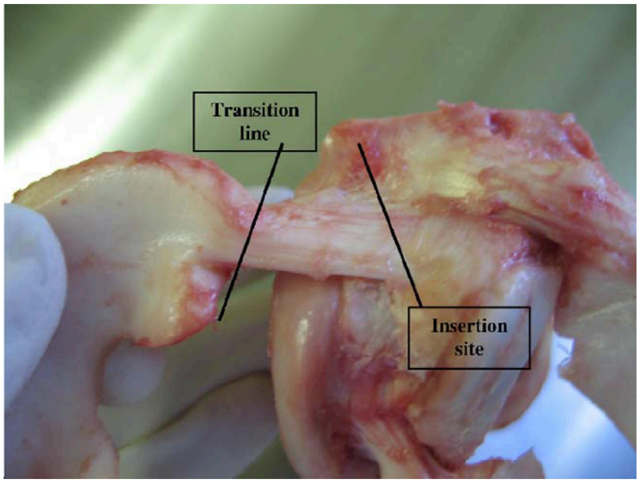

Fig. 3. Superior view of the knee demonstrating the transition from the attachment to the meniscus and the bone insertion site. The collagen fiber orientation of the attachment is also visible.

(Fig. 3). The medial meniscus was bisected allowing the medial anterior attachment (MA) and medial posterior attachment (MP) to be tested. The lateral anterior attachment (LA) was also tested. The posterior attachment of the lateral meniscus inserts into the femur and thus was not included in the current study.

2.2. Mechanical testing

The meniscus was gripped in the clamp at the transition line to the attachment and the tibia was held rigid by the fixture. After equilibrium and alignment were established, each attachment was preconditioned for 10 cycles at 10 mm/min, between 0% and 3% of the gauge length using a sine wave (Sabiston et al., 1990). Immediately following preconditioning, a stress relaxation test was performed. The stress relaxation tests were conducted by ramping up to a deformation of 3% of the gauge length in 1 s and holding for 45 min or until the load did not change by more than 0.5% of the peak load over 1 min (Lam et al., 1995; Haut and Haut, 1997; Thornton et al., 1997; Haut Donahue et al., 2001; Hingorani et al., 2004). In order to utilize the same attachments for both stress relaxation and creep testing, the samples needed to fully recover from the stress relaxation testing. To document the recovery period of the attachments, a pilot study was conducted. Recovery was tested at 12, 24 and 45 h. At all time points the attachment showed full recovery, with changes of approximately 1% in the stress relaxation properties. A 24 h recovery period was used for the tests, since all time points investigated showed full recovery of properties. This data does not indicate how long it takes for recovery, as that time period may be less than 12 h. Specimens were wrapped in saline-soaked gauze prior to being refrigerated overnight at 4 °C. Because the pilot study showed that the attachments recovered after the stress relaxation test in less than 24 h, creep testing was performed on the same attachments the following day. The creep tests were conducted immediately after preconditioning by ramping up to the peak load measured during the stress relaxation test (Hingorani et al., 2004). The load was held constant for 45 min or until the displacement did not change by more than 0.5% of the initial displacement over 1 min.

Three attachments were tested from each specimen. After testing the first attachment, the specimen was repositioned within the fixture, and the next attachment was tested. The attachments were tested in a random order each day.

2.3. System compliance

To measure the compliance of the custom fixture and custom clamp designed for this experiment, a video dimension analysis system was used. Preliminary stress relaxation and creep tests were conducted, following the same protocol outlined above, to determine the compliance of the fixture and the clamp, as well as any slippage of the sample within the grip. A charge-coupled video camera (model MicroPix M-1024, CCD-Direct, Ann Arbor, MI) captured images via a Firewire adapter card. Tissue slippage was examined by tracking a line that marked the location of the bottom of the clamp on the tissue surface. The custom fixture was deemed non-compliant as long as no component deflected further than 0.5% from its original position. The custom clamp was deemed non-compliant if no tissue slippage occurred.

2.4. Data analysis

To characterize the time dependent stress relaxation behavior, the load relaxation rate (N/ln(s)), load at the end of the test (N), normalized relaxation rate (1/ln(s)), normalized load at the end of the test, stress relaxation rate (MPa/ln(s)) and stress at the end of the test (MPa) were determined (Haut Donahue et al., 2001). To normalize the stress relaxation data, the loads were divided by the maximum load value reached during the test.

To quantify the time dependent creep behavior, the displacement creep rate (mm/ln(s)), displacement at the end of the test (mm), normalized creep rate (1/ln(s)), normalized displacement at the end of the test, strain creep rate (1/ln(s)) and the strain at the end of the test (mm/mm) were determined (Haut Donahue et al., 2001). To normalize the creep data, the displacements were divided by the initial displacement of the sample.

2.5. Statistical analyses

Averages and standard deviations were computed for the geometric properties (cross-sectional area, length), and time dependent material properties for each of the three attachments. One-way analysis of variance (ANOVA) was performed using Statview (SAS Institute Inc., Cary, NC) to make comparisons between the three attachments. When significant results were identified by ANOVA, post hoc comparisons were made using Bonferroni–Dunn's method ($p<0.05/3$). Linear regression was performed on the normalized load versus the natural log of time data to determine the normalized rate of relaxation. Similarly, the normalized displacement versus the natural log of time data were used to determine the normalized rate of creep. A significance level of 0.05 was used for all statistical analyses.

Table 1
Cross-sectional areas and lengths of the three bovine attachments

	Cross-sectional area (mm^2)	Length (mm) superior side	Length (mm) inferior side
LA	69.4±9.6	22.6±1.0*	21.7±1.2
MA	55.6±8.3*,**	19.6±2.1*,**	11.8±0.9**
MP	66.5±5.7	16.3±1.0**	10.0±1.0**

*Denotes property significantly different from MP.
**Denotes property significantly different from LA.

3. Results

The MA attachment had a significantly smaller cross-sectional area than the other two attachments ($p \leqslant 0.013$), and all three attachments had significantly different lengths, with the MP attachment being the shortest ($p<0.0004$)(Table 1). For the medial attachments (anterior and posterior) the inferior surface was approximately 40% shorter than the superior surface, whereas for the lateral anterior attachment, the inferior length was about 5% shorter than the superior length (Fig. 6).

The results of the stress relaxation tests can be seen in Table 2. The relaxation rates and normalized relaxation rates had correlation coefficients of 0.97 or higher for the individual attachments (Fig. 4). While not significant, the LA attachment exhibited higher load and stress at the end of the stress relaxation test compared to the other two attachments (∼30%). No significant differences were seen for any of the relaxation rates (load relaxation rate, normalized relaxation rate and stress relaxation rate) between the various attachments.

The creep rates and normalized creep rates had correlation coefficients of 0.98 or higher for the individual attachments (Fig. 5). The percent difference between the various attachments for all the properties presented ranged from approximately 4% to over 45%. In contrast to the stress relaxation properties, significant differences existed between the attachments for the measured creep parameters. Most notably, the LA, which relaxed the least amount, also crept the least. The normalized displacement and strain at the end of the

Table 2
Stress relaxation properties for the lateral anterior (LA), medial anterior (MA) and medial posterior (MP) meniscal horn attachments

Stress relaxation data

	Load at end (N)	Load relaxation rate (N/ln(s))	Normalized load at end	Normalized relaxation rate (1/ln(s))	Stress at end (MPa)	Stress relaxation rate (MPa/ln(s))
LA	30.14±10.00	−10.86±1.56	0.348±0.083	−0.129±0.022	0.438±0.151	−0.158±0.021
MA	21.17±8.01	−9.93±2.10	0.281±0.068	−0.135±0.006	0.381±0.136	−0.179±0.038
MP	21.48±6.30	−9.80±2.14	0.314±0.039	−0.144±0.006	0.328±0.110	−0.148±0.035
Mean	24.26±8.95	−10.20±1.92	0.314±0.069	−0.136±0.014	0.382±0.135	−0.162±0.034

No significant differences present ($p>0.05$).

creep test for the LA attachment was significantly smaller than the MP attachment ($p = 0.0003$) and MA attachment ($p = 0.0117$), respectively. The LA attachment also had a significantly smaller normalized creep rate and strain creep rate compared to the MP attachment ($p \leqslant 0.0043$). The MA attachment and the MP attachment demonstrated no significant differences for any of the creep properties measured ($p > 0.05$) (Table 3).

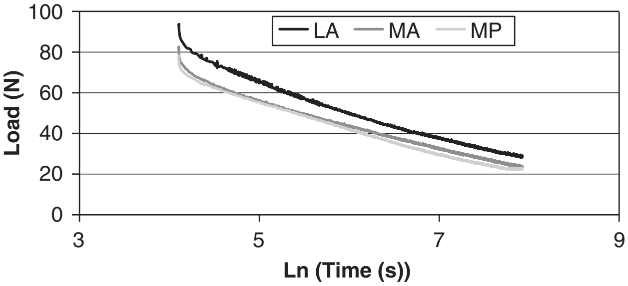

Fig. 4. Sample load relaxation plot illustrating the similarities of the load relaxation rates. Load relaxation rates were determined as the slope of the lines. The load relaxation rates of all three attachments showed no significant differences.

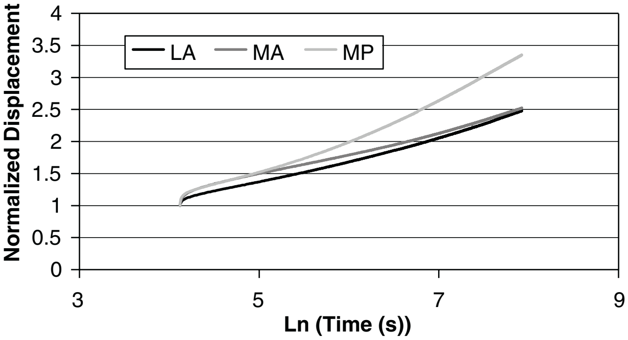

Fig. 5. Sample normalized creep plot demonstrating the differences of the normalized creep rates. The normalized creep rate was determined as the slope of the lines. The normalized creep rate of the MP attachment was significantly larger than the normalized creep rate for the LA attachment.

4. Discussion

The results of this study indicated that the material properties of the meniscal attachments were time dependent in nature, and that their geometric properties varied amongst the attachments. The stress relaxation behavior of the three attachments demonstrated no significant differences. Therefore, to fabricate a successful meniscal replacement, the stress relaxation properties of the horn attachments could be the same. Unfortunately, the creep characteristics exhibited significant differences in several areas, with the LA attachment seemingly different than the MA and MP for various creep parameters. Based on these measurements, a meniscal prosthesis would likely require that the lateral and medial attachments have different creep properties.

4.1. Limitations of study

Although the results indicate that the medial attachments of a meniscal replacement should have one set of time dependent properties while the lateral attachments possess a different set, it is unknown how attachment properties affect knee joint behavior. Previously, a validated finite element model (Haut Donahue et al., 2003) demonstrated that the stiffness of the meniscal attachments affected the contact mechanics of the tibial plateau. However, the attachments were modeled as linearly elastic. Therefore, our future work will address how the range of stress relaxation and creep parameters presented in the current study will affect the contact behavior of the knee.

The profile of the meniscal attachments is wedge-shaped, and the superior side of the attachment is longer than the inferior side of the attachment (Fig. 6). The length measurements presented, and the gauge lengths

Table 3
Creep properties for the lateral anterior (LA), medial anterior (MA) and medial posterior (MP) meniscal horn attachments

Creep data						
	Displacement at end (mm)	Displacement creep rate (mm/ln(s))	Normalized displacement at end	Normalized creep rate (1/ln(s))	Strain at end (mm/mm)	Strain creep rate (1/ln(s))
LA	1.68 ± 0.29	0.267 ± 0.072	2.56 ± 0.33*	0.403 ± 0.094*	0.074 ± 0.012	0.012 ± 0.003*
MA	2.00 ± 0.52	0.313 ± 0.069	2.90 ± 0.27	0.461 ± 0.072	0.103 ± 0.029**	0.016 ± 0.004**
MP	1.61 ± 0.28	0.278 ± 0.053	3.17 ± 0.24	0.549 ± 0.061	0.099 ± 0.017	0.017 ± 0.003
Mean	1.76 ± 0.40	0.286 ± 0.065	2.88 ± 0.37	0.471 ± 0.096	0.092 ± 0.024	0.015 ± 0.004

*Denotes property significantly different from MP.

**Denotes property significantly different from LA.

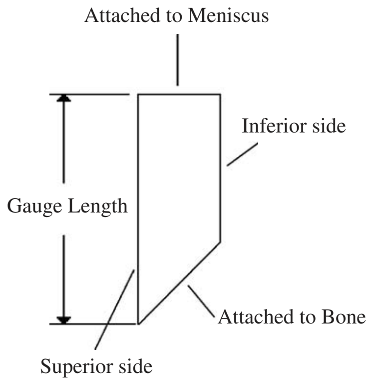

Fig. 6. Profile of the meniscal attachment. The increased length of the superior side versus the inferior side creates a wedge like profile. The testing displacements were based on the length of the superior side only. Table 1 documents the lengths of both the superior and inferior sides.

used to calculate the testing displacements for the experiment, were based on the length of the superior side of the attachment solely. If we were to repeat the data analysis and use the average length instead to compute the strain at the end or the strain creep rate, this would only amplify the differences, because the lateral attachment would stay essentially the same length, and the medial attachments would be effectively shorter, further increasing the strains, and the differences between the lateral and medial attachments would be amplified.

4.2. Significance of results

It is hypothesized that the geometric properties of the meniscal attachments reflect upon their motion and functionality within the knee. The results of this study indicated that the geometric properties of the meniscal horns vary amongst the attachments. Not only does the bovine LA creep less and relax less than the other two attachments, it has a larger cross-sectional area. It has previously been shown that in the human knee joint the LA attachment displaces more during joint loading than its medial counterpart, and the lateral meniscus is exposed to higher pressures than the medial meniscus, which in turn leads to larger tensile hoop stresses being generated within the lateral meniscal tissue and attachments (Walker and Erkman, 1975; Shrive et al., 1978). While it is rare to see meniscal horn attachment failure during in vivo loading, it is likely that the material properties control the overall motion of the meniscus, and thus further studies will determine if these differences in properties between the lateral and medial meniscal attachments would correlate to changes in overall meniscal motion. It is also believed that the posterior attachments of the menisci are exposed to both tensile and compressive forces during knee flexion and extension (Gao et al., 1994; Gao and Messner, 1996; Goertzen et al., 1996a, b; Gao et al., 1998; Messner and Gao, 1998). No differences were noted between the medial anterior and posterior attachments for either the stress relaxation or creep properties, but the cross-sectional area and lengths were different. One might expect that changing the attachments loading environment from tension-only to tension–compression may result in changes in tissue organization and morphology. Current studies are underway documenting the meniscal attachment histology. It is important to note that the meniscal motions and loading environment noted above are from rabbit and human data. This study was conducted on bovine menisci where, in contrast to human menisci, the posterior attachment of the lateral meniscus inserts solely into the femur. The use of a bovine knee model is a common practice due to their availability from a local abattoir, and bovine tendon properties have been shown to correlate well to human tendon properties (Haut Donahue et al., 2001). Future work should look at the structural and material properties of human meniscal attachments.

The fact that the MP attachment elongates more rapidly than both anterior attachments could stem from the shorter overall length of the posterior attachment compared to the anterior attachments. For the posterior attachment to undergo comparable displacements to the anterior attachments, it must strain to a greater extent. Thus, to allow for this greater percent elongation, the strain creep rate of the posterior attachment must increase. Also, the normalized displacement at the end of the test showed significant differences between the LA attachment and the MP attachment. Therefore, under constant load conditions, the LA attachment would resist elongation to a greater extent than the MP attachment. This was evident from the significantly smaller normalized creep rate and normalized displacement at the end of the test compared to the posterior attachment.

The clinical implications of these results indicate that when designing and implementing a meniscal prosthesis, the horn attachments cannot possess the same time dependent properties. If the time dependent properties of the meniscal attachments on the prosthesis were the same, the biomechanical function of the knee may be compromised. Since some attachments elongate to a greater extent than others, designing a replacement with the same creep properties for every attachment may lead to greater displacements of the replacement meniscus within the joint space. This would result in a greater joint laxity and reduce the ability of the meniscus to reestablish joint congruency. By increasing the joint laxity and reducing the congruency effects of the meniscus, other injuries may arise within the knee. The articular cartilage of both the femoral condyles and the tibial plateau would be exposed to altered stresses, which could result in their progressive degeneration and, ultimately, osteoarthritis. Therefore, a successful meniscal replacement should consider independent sets of properties for the horn attachments.

The findings of this study help document the time dependent properties of the meniscal horn attachments, as well as the variation in properties of these attachments. These parameters, along with meniscal tissue material properties, are important in the development of a successful meniscal replacement. This paper is the first step into comprehending and quantifying the properties of meniscal attachments. Future studies of failure characteristics are needed to gain a complete understanding of the properties these attachments possess.

Acknowledgements

This work was funded by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR051906-01).

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