Comparison of Viscoelastic, Structural, and Material Properties of Double-Looped Anterior Cruciate Ligament Grafts Made From Bovine Digital Extensor and Human Hamstring Tendons

Tammy L. Haut Donahue

Colin Gregersen

M. L. Hull[1]

Biomedical Engineering Program,
University of California at Davis,
Davis, CA 95616

Stephen M. Howell

Department of Mechanical Engineering
and Biomedical Engineering Program,
University of California at Davis,
Davis, CA 95616

Due to ready availability, decreased cost, and freedom from transmissible diseases in humans such as hepatitis and AIDS, it would be advantageous to use tendon grafts from farm animals as a substitute for human tendon grafts in in vitro experiments aimed at improving the outcome of anterior cruciate ligament (ACL) reconstructive surgery. Thus the objective of this study was to determine whether an anterior cruciate ligament (ACL) graft composed of two loops of bovine common digital extensor tendon has the same viscoelastic, structural, and material properties as a graft composed of a double loop of semitendinosus and gracilis tendons from humans. To satisfy this objective, grafts were constructed from each tissue source. The cross-sectional area was measured using an area micrometer, and each graft was then pulled using a materials testing system while submerged in a saline bath. Using two groups of tendon grafts ($n=10$), viscoelastic tests were conducted over a three-day period during which a constant displacement load relaxation test was followed by a constant amplitude, cyclic load creep test (first day), a constant load creep test (second day), and an incremental cyclic load creep test (third day). Load-to-failure tests were performed on two different groups of grafts ($n=8$). When the viscoelastic behavior was compared, there were no significant differences in the rate of load decay or the final load (relaxation test) and rates of displacement increase or final displacements (creep tests) ($p>0.115$). To compare both the structural and material properties in the toe region (i.e., <250 N) of the load-elongation curve, the tangent stiffness and modulus functions were computed from parameters used in an exponential model fit to the load (stress)—elongation (strain) data. Although one of the two parameters in the functions was different statistically, this difference translated into a difference of only 0.03 mm in displacement at 250 N of load. In the linear region (i.e., 50–75 percent of ultimate load) of the load-elongation curve, the linear stiffness of the two graft types compared closely (444 N/mm for bovine and 418 N/mm for human) ($p=0.341$). At failure, the ultimate loads (2901 N and 2914 N for bovine and human, respectively) and the ultimate stresses (71.8 MPa and 65.6 MPa for bovine and human, respectively) were not significantly different ($p>0.261$). The theoretical effect of any differences in properties between these two grafts on the results of two types of in vitro experiments (i.e., effect of surgical variables on knee laxity and structural properties of fixation devices) are discussed. Despite some statistical differences in the properties evaluated, these differences do not translate into important effects on the dependent variables of interest in the experiments. Thus the bovine tendon graft can be substituted for the human tendon graft in both types of experiments. [DOI: 10.1115/1.1351889]

1 Introduction

Because tendons are used commonly as grafts in many orthopedic surgical procedures (e.g., reconstruction of a torn anterior cruciate ligament), the need exists for an abundant supply for experimental research in vitro aimed at improving surgical outcome. However, human tendons from young donors are scarce, expensive, and can transmit diseases including hepatitis and acquired immune deficiency syndrome. Readily available, inexpensive, and free from the transmissible diseases above in humans, tendons from farm animals (e.g. bovine, porcine, ovine) could be used as a substitute for human tendons during in vitro experiments if the viscoelastic, structural, and material properties are shown to be similar.

The interest in this paper is on an animal substitute for a double-looped semitendinosus and gracilis (DLSTG) graft as a replacement for a torn anterior cruciate ligament (ACL). Since the DLSTG graft is becoming more widely used in clinical practice

[1]Send all correspondence to Professor M. L. Hull, Tele: 916-752-6220; Fax: 916-752-4158; E-mail: mlhull@ucdavis.edu.

Contributed by the Bioengineering Division for publication in the JOURNAL OF BIOMECHANICAL ENGINEERING. Manuscript received by the Bioengineering Division Oct. 1999; revised manuscript received Oct. 2000. Associate Editor: L. J. Soslowsky.

 Copyright © 2001 by ASME

[1,2], improvements in clinical outcome may be possible if the surgical variables that govern the load-displacement behavior of the knee are systematically investigated in cadaveric knees reconstructed with double-looped grafts. Among the specific topics of interest that may influence clinical outcome are the structural properties of both tibial and femoral fixation devices [3–5] and the combination of fixation device stiffness and graft pretension that best restores normal knee kinematics [6]. Because the viscoelastic, structural, and material properties of the graft can affect the results of experiments that investigate these topics, all three properties should be investigated in any comparative study of animal and human tendon grafts.

No previous study known to the authors has compared the viscoelastic, structural, and material properties of human and animal tendons configured as a double-looped graft for replacing a torn ACL. Only the structural properties and not the material and viscoelastic properties of the human double-looped semitendinosus and gracilis graft have been measured [5,7]. The viscoeleastic properties have been determined for single animal tendons including swine and canine [8] and porcine [9] tendons. The structural and material properties have also been determined for single bovine [10,11] and kangaroo [12] tendons treated with glutaraldehyde to be used as xenografts in humans. However, none of these studies has fully characterized the viscoelastic, structural, and material properties of either human or animal tendons configured as a double-looped ACL graft.

Considering both that the need exists to identify an animal tendon to substitute for a human DLSTG graft for in vitro experiments and that no previous study has made such an identification, the objective of this study was to determine whether a tendon graft for the ACL composed of two loops of bovine tendon has the same viscoelastic, structural, and material properties as a human DLSTG graft.

2 Methods and Materials

2.1 Experiments. Four groups of grafts were used to determine viscoelastic, structural, and material properties. Two groups of grafts (one animal and one human) were used in viscoelastic tests ($n=10$ in each group) and two separate groups of grafts (one animal and one human) were used in the load-to-failure tests ($n=8$ in each group) in which structural and material properties were determined. For both types of tests, animal and human grafts were prepared from fresh-frozen tissue stored at $-20°C$. The grafts using animal tissue were constructed from the common digital extensor tendons harvested from bovine forelimbs of different skeletally mature animals (age$\approx$2 years). The double-looped bovine tendon (DLBT) graft was prepared by dividing the bifurcating tendon into two halves and excising nontendinous tissue. A #1 suture (Ethibond, Ethicon Inc., Somerville, NJ) was used to sew 4 cm of both ends of each tendon using a crisscrossing stitch. A graft was made by placing the two tendons side-by-side and folding them in half (Fig. 1). The cross-sectional area of the DLBT graft was calculated by averaging cross-sectional area measurements obtained at three equal increments from the looped end of the graft using an area micrometer [13,14].

The grafts using human tissue were constructed from gracilis and semitendinosus tendons. For the viscoelastic tests, the average age of the donors was 56 years (range 22–78 years) and for the load-to-failure tests the average age was also 56 years (range 46–67 years). The preparation and measurement of the cross-sectional area of the double-looped semitendinosus and gracilis grafts were similar to the technique used for the double loop bovine tendon graft.

To evaluate the viscoelastic, structural, and material properties, load-elongation tests of the grafts were administered using a materials testing machine (MTM) (Model 858, MTS Corporation, Minneapolis, MN). Tensile loads were measured with a 13.3 kN load cell attached to the base of the machine. Each graft was installed in the MTM by looping it around a bar 6.35 mm in diameter attached to the base, evenly tensioning the four limbs of the graft, and clamping them with a freeze clamp bolted to the crosshead of the MTM. Even tensioning was accomplished with a special jig that applied a 5 N weight to each limb (Fig. 2). The distance between the bar and freeze clamp was adjusted differently depending on the shortest length of the tendons available in a particular group. For the ten bovine tendon grafts and the ten human tendon grafts used in the viscoelastic tests, the grip-to-grip distance was 85 mm and 75 mm, respectively. For the eight bovine tendon grafts and the eight human tendon grafts used in the load-to-failure tests, the grip-to-grip distance was 95 mm. This difference in lengths was necessary to allow sufficient graft material to interface with the freeze clamp. Elongation between the freeze clamp and the bar was measured with the linear variable differential transformer integrated into the MTM. With the exception of the freeze clamp, the bar and the graft were immersed in a buffered saline bath at room temperature. A computer was used both to control the tests and to acquire load and elongation data (Multipurpose Testware, MTS Corporation, Minneapolis, MN).

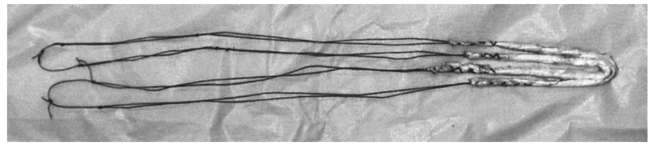

Fig. 1 Photograph of a double–looped graft with the sutures attached to each of the four limbs

Viscoelastic tests were performed over a three-day period using the same two groups of grafts (Fig. 3). The testing on the first day approximated the loading in in vitro tests that have been used to evaluate the effect of independent variables (e.g., tibial tunnel position) on the anterior load-displacement behavior of the ACL-reconstructed knee [15]. Each graft was first preconditioned for ten cycles at 0.1 Hz between 20 N and 250 N. After a wait period of 15 minutes, a load of 20 N was applied to establish the initial gage length and the graft was then stretched to 2.5 percent strain at a rate of 250 mm/s. The 2.5 percent strain value corresponded to a load of approximately 500 N. The final displacement was held constant while the declining load was measured at 4 Hz either for 15 minutes or until the load changed less than 0.1 percent over 1 minute. After another 15-minute wait period, a cyclic creep test was conducted. The tendon was cycled at 1 cycle/min between 20 N and 225 N until less than a 0.1 percent change in displacement was seen for three successive cycles. Following the tests, each tendon was placed in a saline bath and refrigerated overnight.

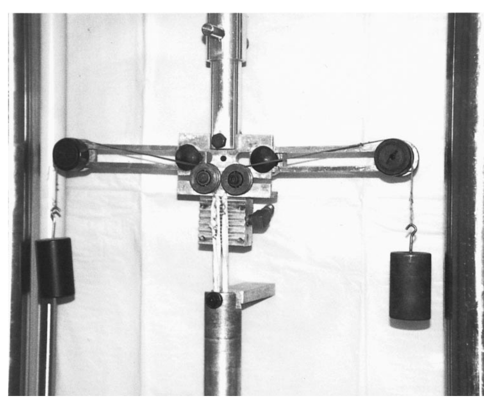

Fig. 2 Photograph of the test set up illustrating the jig used to equally tension all four limbs of the double–looped graft. The saline bath and the other half of the freeze clamp have been omitted for clarity.

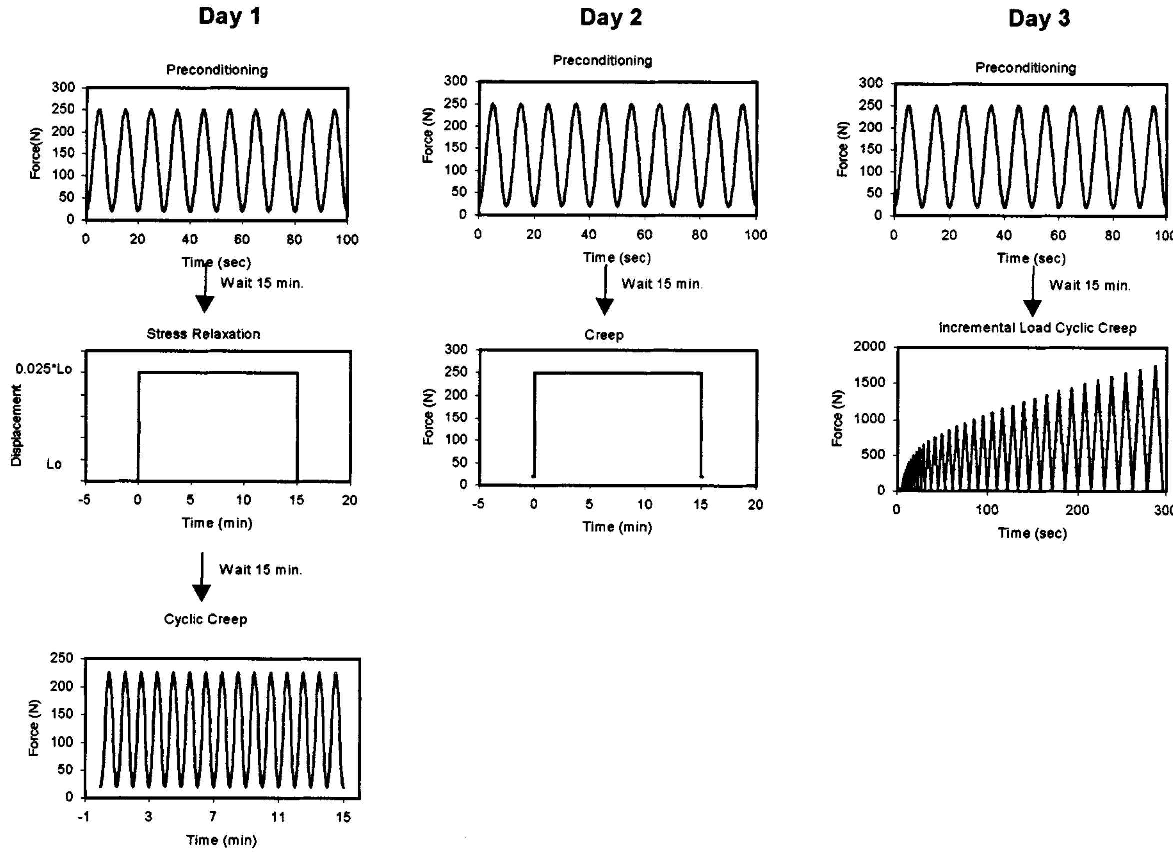

Fig. 3 Plots illustrating the history for the viscoelastic tests over the three-day test period

Because a pilot study showed that the tendon recovered after 24 hours (refer to Section 4), further testing was performed on the same tendon on subsequent days. On the second day, a constant load creep test was performed to compare the creep behavior of grafts constructed from the two materials. Following the same preconditioning and 15-minute wait period as on day one, the graft was loaded at a rate of 315 N/s. The load was increased from 20 N to 250 N and held constant while the displacement was monitored at 1 Hz either for 15 minutes or until the displacement changed less than a 0.1 percent over 1 minute. Following the test, again the tendon was stored overnight as described above.

Since the pilot testing also showed that the tendon recovered from the second day of testing following another 24 hours, testing was extended into the third day to simulate incremental load tests that have been used to evaluate fixation device slippage [4]. After preconditioning and the 15-minute wait period as on the previous two days, the tendon was cycled between 25 N and 1750 N in 50 N increments (35 cycles total) at a rate of 200 N/s. With this loading protocol, the first cycle was from 25 N to 50 N, the second was from 25 N to 100 N, and so on. In this test the displacement was sampled at 20 Hz.

To characterize the structural and material properties beyond the toe region, load-to-failure tests were performed using two different groups of grafts from those used in the viscoelastic tests. Each graft was preconditioned as described above and then pulled to failure at a strain rate of 2 percent/s following a 15-minute wait period (Fig. 4). This relatively slow strain rate was selected so that the viscoelastic behavior of the graft did not affect the load-elongation behavior in the load-to-failure tests [16]. As with the viscoelastic tests, an initial tension of 20 N was applied to determine the gage length.

2.2 Data Analysis. The viscoelastic behaviors between bovine and human double-looped tendon grafts were compared using the results from the constant displacement relaxation tests, the constant amplitude cyclic load creep tests, the constant load creep tests, and the cyclic incremental load creep tests. For the constant displacement relaxation test, the results were analyzed by plotting both the actual load versus the $\ln(t)$ and the stress normalized to the stress immediately after the initial 2.5 percent strain had been applied (i.e., stress at time zero) versus the $\ln(t)$. The logarithmic operation on the time axis transformed the inherently nonlinear relation in load decay versus time (Fig. 5) into a linear relation in the load decay versus $\ln(t)$ so that a simple linear regression could be used to determine the rates of relaxation. The average load and

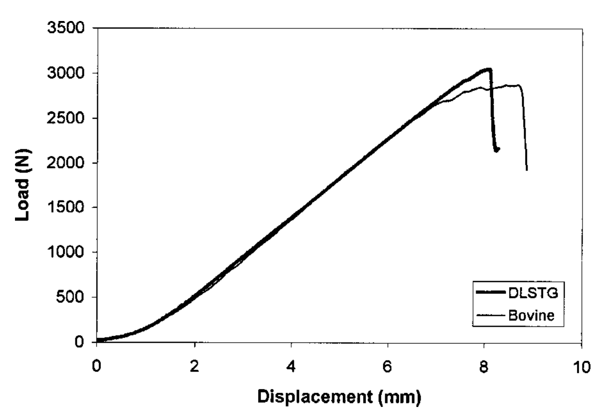

Fig. 4 Example plot comparing the load-displacement of the bovine and human grafts from the load-to-failure test. Inasmuch as all grafts failed by midsubstance tears, the clamping procedures did not influence the determination of the ultimate load.

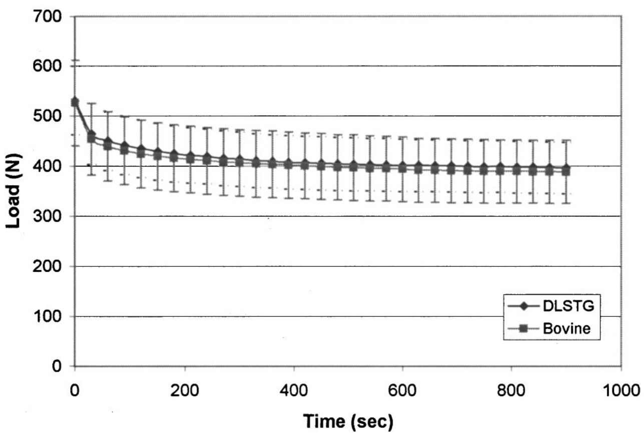

Fig. 5 Average and 95 percent confidence limits of the load versus time for double–looped bovine and human tendon grafts in the constant displacement relaxation test with initial strain of 2.5 percent ($n=10$). After the 15 minute relaxation period, the load difference between the two tendon grafts was not statistically significant ($p=0.894$).

the average normalized stress relaxation rates for the bovine and human grafts were compared using an unpaired t-test. Also both the average load and the average normalized stress at the end of the relaxation were tested for statistically significant differences using an unpaired t-test.

Because a pilot study showed that graft displacement was inversely related to the length of the graft (refer to Section 4), the displacement data from the three creep tests were analyzed by first scaling the displacement of the bovine tendon grafts inversely with length to produce an equivalent displacement corresponding to the 75 mm length of the human tendon grafts. Both the residual displacement and the strain at the valley of each cycle were computed from the difference between the length at the cycle of interest and the original length. Throughout Section 2.2, all strains were calculated based on elongation measured between the two grips. Final values of both residual displacement and strain were tested for significant differences using an unpaired t-test.

The data from the constant load creep test were analyzed similarly to the constant displacement relaxation test. First, the scaled displacement of the bovine tendon grafts was computed as described above. The normalized strain was computed as the ratio between the strain at an arbitrary time to the strain at the time that the constant load was reached (i.e., time zero). Both the rate of displacement and the rate of normalized strain were determined by plotting both quantities versus $\ln(t)$ and then computing the slopes using simple linear regression. The rates for the two types of graft materials were compared using an unpaired t-test. Also the displacement and the normalized strain at the end of the test were compared using the same type of analysis.

Structural and material properties in the toe region defined by a 250 N load limit were determined from the load-elongation curve recorded during the application of the 1000 N load cycle in the incremental creep test performed on the third day of viscoelastic testing. In the toe region, the slope B and intercept parameter C of the tangent stiffness function (i.e., $dF/d\delta=BF+C$) were computed by fitting the exponential load-displacement model (i.e., $F=A(e^{B\delta}-1)$ where F is the force, δ is the displacement, A,B are constants, and $C=AB$) to the experimental data using nonlinear regression. The slope and intercept parameters of the tangent modulus function were also computed by substituting stress for the force F and strain for the displacement δ and then performing the same operations using the stress-strain data.

Structural and material properties beyond the toe region were determined from the load-elongation curve recorded in the load-to-failure tests. In the linear region defined as the region between 50 and 75 percent of the failure load, the stiffness was computed using simple regression. The tensile modulus was then computed by multiplying the measured stiffness by the graft gage length and dividing the product by the average cross-sectional area of the graft. The ultimate load, ultimate stress, ultimate displacement, and ultimate strain were also computed. All of the structural and material properties determined from the load-to-failure test were compared statistically using unpaired t-tests for each property. Differences were considered significant when $p<0.05$.

3 Results

There were no significant differences in any of the measures of viscoelastic behavior between the bovine and human tendon grafts for any of the four tests. During 15 minutes of constant displacement in the relaxation test, the average R-squared value of the load relaxation rate was 0.975 (range 0.932–0.998) and the load relaxation rates for the two types of grafts differed by 0.2 N/ln(s) ($p=0.945$) (Table 1). At the end of the test the load difference was only 5 N ($p=0.894$) (Fig. 5). During the cyclic creep test immediately following the constant displacement load-relaxation test (Fig. 6), the residual displacement difference was only 0.03 mm at steady state ($p=0.360$). During the constant load creep tests, the average R-squared value for the rate of creep displacement was 0.981 (range 0.957–0.992) and the rate of creep displacement for the bovine tendon graft [0.041mm/ln(s)] was similar to that for the human tendon grafts [0.038mm/ln(s)] ($p=0.381$) (Fig. 7). Not surprisingly, the creep displacements at the end of the tests were also similar differing by only 0.03 mm ($p=0.115$) (Table 2). During the incremental creep test on the third day of testing, the incremental residual displacements were virtu-

Table 1 Summary of viscoelastic quantities (mean±S.D.) from the relaxation test. The constant displacement was 2.5 percent strain. None of the quantities was different between the bovine and human tendon grafts.

Tissue	Load Relaxation Rate (N/ln(s))	Load at End (N)	Normalized Stress Relaxation Rate (1/ln(s))	Normalized Stress at End
Bovine	-19.98 ± 5.45	388 ± 89	-0.038 ± 0.007	0.741 ± 0.043
Human	-19.76 ± 8.32	393 ± 63	-0.036 ± 0.013	0.758 ± 0.0560
p-value	0.945	0.894	0.750	0.488

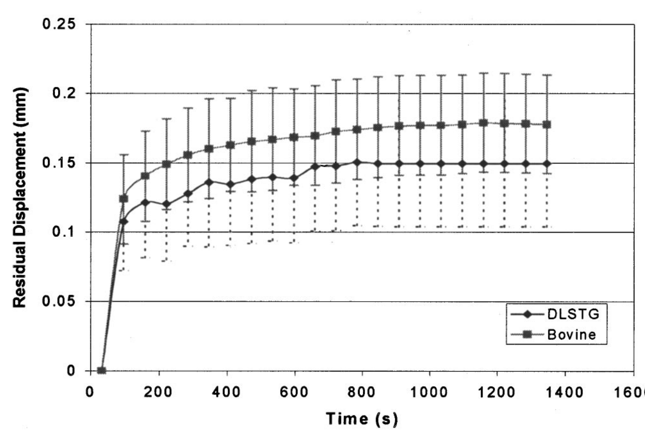

Fig. 6 Average and 95 percent confidence limits of the residual displacement versus time for double–looped bovine and human tendon grafts in the constant load cyclic creep test ($n=10$). Following the 15 minute creep period, the difference between the two tendons was not statistically significant ($p=0.360$).

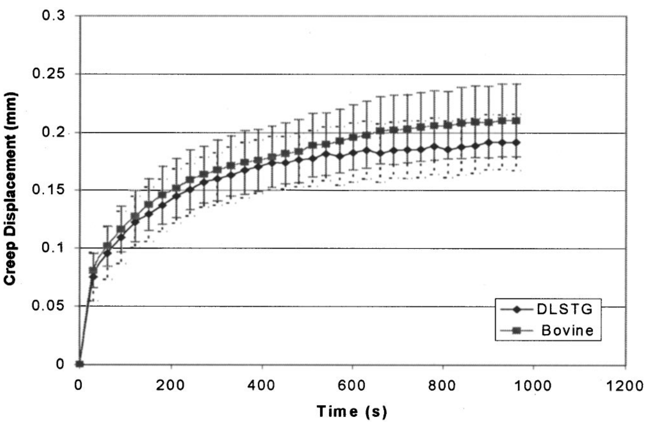

Fig. 7 Average and 95 percent confidence limits of the creep displacement versus time for double–looped bovine and human tendon grafts in the constant load creep test ($n=10$). Following the 15 minute creep period, the difference between the two tendons was not statistically significant ($p=0.115$).

ally identical throughout the test (Fig. 8) and the final displacements differed by only 0.04 mm ($p=0.762$) (Table 3).

For the tangent stiffness and modulus functions computed from the toe region of the load-elongation curve in the incremental creep test, one parameter was different between the two graft types but the other parameter was the same. The average cross-sectional areas between the two grafts compared closely (38.9 mm^2 for bovine and 41.4 mm^2 for human) and were not significantly different ($p=0.280$) (Table 4). The minimum R-squared value for the nonlinear regression used to compute the tangent stiffness and modulus parameters was 0.999 indicating that the exponential model was appropriate up to the 250 N load limit. The tangent stiffness intercept parameters were the same for the bovine tendon grafts (140.1 N/mm) and the human tendon grafts (145.8 N/mm) ($p=0.557$) while the tangent stiffness slope parameters were different (1.21/mm for bovine and 1.04/mm for human) ($p=0.037$). Because the cross-sectional areas of the two types of grafts were comparable, the results for the tangent modulus parameters mirrored those for the tangent stiffness parameters.

Of the structural and material properties determined in the load-to-failure tests, all were the same except for the tensile modulus in the linear region (Table 5). The average cross-sectional areas between the two grafts compared closely (40.9 mm^2 for bovine and 44.4 mm^2 for human) and were not significantly different ($p=0.275$) (Table 5). The minimum R-squared value for the linear regression used to compute the linear stiffness and modulus was 0.999 indicating that the linear model was appropriate between 50 and 75 percent of the ultimate load. The linear stiffnesses also compared closely with the linear stiffness of the bovine tendon graft (444 N/mm) being only about 5 percent greater than that of the human tendon grafts (418 N/mm) ($p=0.341$). Because the cross-sectional area of the bovine grafts was somewhat smaller than that of the human tendon grafts whereas the linear stiffness of the bovine tendon grafts was somewhat greater than that of the human tendon grafts, the linear tensile modulus of the bovine tendon grafts (1033 MPa) exceeded that of the human tendon grafts (904 MPa) ($p=0.010$). The average ultimate loads were similar (2901 N and 2914 N for the bovine and human grafts, respectively) ($p=0.960$) as were the average ultimate stresses (71.8 MPa for bovine and 65.6 MPa for human) ($p=0.261$). Likewise both the ultimate displacements (8.6 mm bovine and 8.4

Table 2 Summary of viscoelastic quantities (mean±S.D.) from the constant load creep test. The constant load was 250 N. None of the quantities was different between the bovine and human tendon grafts.

Tissue	Displacement Creep Rate (mm/ln(s))	Displacement at End (mm)	Normalized Strain Creep Rate (1/ln(s))	Normalized Strain at End
Bovine	0.041 ± 0.009	0.22 ± 0.04	0.029 ± 0.007	1.146 ± 0.029
Human	0.038 ± 0.007	0.19 ± 0.03	0.025 ± 0.006	1.131 ± 0.027
p-value	0.381	0.115	0.292	0.229

Table 3 Summary of viscoelastic quantities (mean±S.D.) from the constant amplitude and the incremental amplitude cyclic load creep tests. In the constant amplitude load test, the load was cycled between 20 N and 225 N. In the incremental amplitude load creep test, the load was cycled between 25 N and 1750 N in 50 N steps. None of the quantities was different between the bovine and human tendon grafts.

Tissue	Residual Displacement at End of Constant (mm)	Strain at End of Constant (%)	Residual Displacement at End of Incremental (mm)	Strain at End of Incremental (%)
Bovine	0.18 ± 0.06	0.235 ± 0.076	0.89 ± 0.15	1.180 ± 0.200
Human	0.15 ± 0.07	0.194 ± 0.094	0.93 ± 0.37	1.217 ± 0.482
p-value	0.360	0.308	0.762	0.827

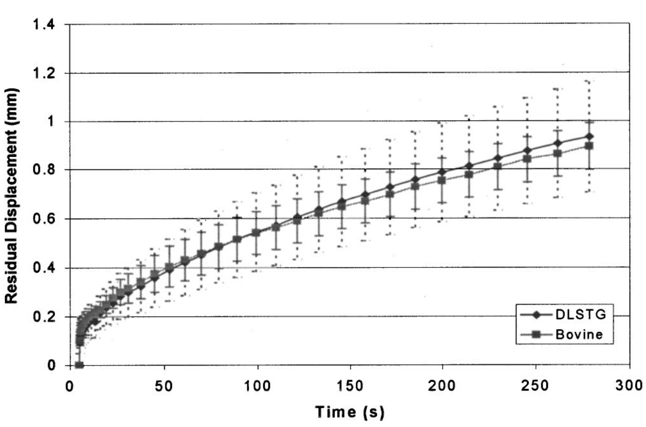

Fig. 8 Average and 95 percent confidence limits of the residual displacement versus time for double–looped bovine and human tendon grafts in the incremental load cyclic creep Test ($n=10$). Following the creep period, the difference between the two tendons was not statistically significant ($p=0.762$).

Table 4 Summary of structural and material properties (mean ±S.D.) for the toe region. Although the tangent stiffness slope parameter was different between the bovine and human tendon grafts, this did not translate into a displacement difference at 250 N of load.

Tissue	Area (mm^2)	Tangent Stiffness Intercept (N/mm)	*Tangent Stiffness Slope (1/mm)	Tangent Modulus Intercept (MPa)	*Tangent Modulus Slope	250 N Displacement (mm)
Bovine	38.9 ± 2.4	140.1 ± 21.6	1.21 ± 0.17	278.5 ± 46.2	92.8 ± 12.9	0.91 ± 0.06
Human	41.4 ± 6.5	145.8 ± 20.1	1.04 ± 0.16	278.8 ± 60.6	79.8 ± 12.0	0.94 ± 0.06
p-value	0.280	0.557	0.037	0.992	0.037	0.314

* denotes properties which were significantly different (p< 0.05).

Table 5 Summary of structural and material properties (mean ±S.D.) from the load-to-failure test. Only the linear modulus was different between the bovine and human tendon grafts.

Tissue	Area (mm^2)	Linear Stiffness (N/mm)	Ultimate Load (N)	Ultimate Displacement (mm)	*Linear Modulus (MPa)	Ultimate Stress (MPa)	Ultimate Strain (%)
Bovine	40.9 ± 5.8	444 ± 66	2901 ± 239	8.6 ± 0.8	1033 ± 71	71.8 ± 9.1	9.0 ± 0.9
Human	44.4 ± 6.7	418 ± 36	2914 ± 644	8.4 ± 1.3	904 ± 99	65.6 ± 12.0	8.8 ± 1.4
p-value	0.275	0.341	0.960	0.687	0.010	0.261	0.687

* denotes properties which were significantly different (p< 0.05).

mm human) ($p=0.687$) and the ultimate strains (9.0 percent bovine and 8.8 percent human) ($p=0.687$) were comparable.

4 Discussion

The goal of this study was to determine whether bovine tendon grafts could be used as a replacement for human tendon grafts in in vitro studies that required the use of a double-looped tendon graft as a replacement for the ACL. The approach taken to satisfy this goal was to compare viscoelastic, structural, and material properties of double-looped grafts composed of both human and bovine tendons. Inasmuch as the experimental techniques have the potential to affect the results and hence their interpretation, a critical examination of these techniques is warranted before delving into the results.

4.1 Methodological Issues. Although the testing methodology did not reflect the manner in which a multi-bundle ACL graft is stressed in a cadaveric knee, it nevertheless was appropriate for the purposes of the study. The knee flexes through a range of motion and stresses the graft in both tension and shear. Because of reciprocating behavior of the anterior and posterior bundles of a DLSTG graft [17], the bundles do not carry equal loads particularly at the extremes of the flexion arc. However, to determine the structural and material properties for comparison purposes, the bundles were equally tensioned before freezing and pulled along the length of the tendon. Accordingly, the bundles experienced only tensile loads and the stress in each bundle was approximately equal. Inasmuch as the construction of the two grafts was identical (i.e., two tendons looped) both in the tests and in a reconstructed knee, a comparison of properties determined in the axial loading tests would be expected to apply to a reconstructed knee. However, the values reported may not necessarily translate to a reconstructed knee where the bundles may not be equally tensioned.

In gripping the specimens to perform the load-elongation tests, techniques were chosen specifically to remove the confounding effects of gripping to the maximum extent possible. If the post around which the tendons were looped deformed, then this would have contributed to the measurement of grip-to-grip displacement. Accordingly to isolate the properties of the grafts per se, the post was designed so that it was at least 100 times stiffer than the graft.

Because the post introduced a local effect by compressing the tendons at the loop point, the effect on the tangent and linear moduli and ultimate strain should be considered. If the compression contributed to the elongation, then both moduli would be less and the ultimate strain would be greater than the corresponding properties of a graft in which the compression effect was absent. The results of a pilot study described below suggest that any contribution from the local compression effect was minimal because the displacement of a tendon graft could be determined by scaling according to length. Scaling according to length would not have yielded accurate displacement values if the compression effect contributed to the elongation. Evidently the preconditioning cycles rendered any contribution to elongation from the compression effect minimal in subsequent loading. Thus both moduli and the ultimate strain are probably representative of those of the graft material per se. Also neither of the clamping methods affected the measurements of the ultimate strength and ultimate stress. This is because the grafts were observed to always fail in the midsubstance and never at the clamps.

Grip-to-grip spacing was chosen for measurement of tissue elongation instead of video dimension analysis (VDA) for three reasons. First, part of the tendon is frozen inside the freeze clamp and unfrozen outside the clamp. There is a distinct line at this junction. This ''freeze-line'' was monitored during the experiments and did not move from the edge of the freeze clamp, indicating that there was no slippage of the tendon. Second, VDA can have very large errors at low strain levels, decreasing to about 10 percent at 10 percent nominal strain [18]. Finally, the site of failure does not correlate with areas of maximum surface strain (as measured with VDA) suggesting that higher strains could exist deeper in the tissue [19]. In consideration of these three reasons, measuring grip-to-grip displacement was more appropriate than measuring the displacement between two lines on the tissue for the purposes of our study. However, it should be recognized that computing strain and other material properties based on grip-to-grip displacements for a collagen-based material such as a double-loop tendon graft represents the average over the whole length of the graft and that local strains and other properties may differ from the average.

To economize on the use of human tendons, the same tendons were subjected to a series of viscoelastic tests over a three-day period. Because viscoelastic response is more pronounced in tendons which are recovered [20] and because any differences may be more pronounced as well, it was of interest to test grafts in a recovered state to obtain a worst case analysis.

Two pilot studies were conducted on four grafts (two bovine grafts and two human grafts) to confirm that grafts were recovered after the 24-hour wait period. To confirm that the grafts recovered from day one to day two of the testing protocol, in one pilot study grafts were subjected to the same protocol used on day one and then the same tests on day one were repeated including preconditioning but excluding the constant amplitude load cyclic creep test. To confirm that the grafts recovered from day two to day three, in the second pilot study grafts were subjected to the same protocol as on day one and day two and on day three the tests on day two were repeated (i.e., preconditioning followed by constant load creep). The root mean squared error (RMSE) between the last cycles of preconditioning, the load versus time plots of the stress relaxation test, and the displacement versus time plots for the creep tests were computed for each of the four grafts tested. The RMSE in load for the preconditioning cycles was less than 1 N, the RMSE for the stress relaxation was less than 25 N, and the RMSE for the constant load creep was equal to 0.1 mm indicating that the grafts recovered between the 24-hour wait periods over the three-day testing protocol.

One parameter affecting the structural properties was the length of the graft. Ideally, both grafts would have been the same length so that no adjustment to the structural properties was necessary to make a valid comparison between the two graft types. However, this was not possible in this study since the human grafts were obtained from a variety of sources and the lengths varied considerably. To standardize the length within a tissue type, the length had to be such that a double-looped graft could still be made with the shortest tendon. The required length for the human tendon grafts was 75 mm for the viscoelastic tests and 95 mm for the load-to-failure tests.

Although the graft lengths for the two graft materials were different, the structural properties can still be compared with confidence based on the results of a pilot study. In the pilot study, the displacements of the same graft at two different graft lengths (85 mm and 75 mm) were measured directly. When the displacement of the longer graft was scaled inversely proportional to the length, the scaled displacement was virtually identical to the measured displacement of the shorter graft.

Although neither of the graft lengths used in the viscoelastic tests corresponded to the length of a typical graft, the structural properties for a standard length graft can still be determined. For a commonly used technique in ACL reconstructive surgery using a DLSTG graft [2], a length of 95 mm is representative. Thus the elongations for a 95 mm length graft can be determined from the data in Tables 1–4 by scaling as noted above.

Because the average age of patients who have an ACL reconstruction is lower than the average age (56 years) of the human tendons used in the experiments herein, the age of the human tendons used in this study should be examined for any potential affect on the results. A previous study determined both the viscoelastic and tensile properties of human patellar tendon for two age groups, one ranging in age from 29–50 years and a second ranging in age from 64–93 years [21]. The only property that was significantly different statistically between the two groups was the ultimate tensile strength. Considering this result in conjunction with the fact that our specimens were younger than those of the older age group used in their study, the age of our specimens should not have affected the measured properties in any substantive manner.

Bovine extensor tendons were a natural choice to form a double-loop graft for three reasons. First they are readily available in cow's feet which can be procured easily and inexpensively from any processor. Second the extensor tendon has the same cross-sectional area (i.e., approximately 40 mm^2) as the pair of human hamstring tendons (Tables 4 and 5). Finally two tendons of comparable cross-section can be formed easily since the tendon is already split naturally along at least half the length.

Of the several nondestructive methods that can be used to measure cross-sectional area [13,22], this study measured this quantity using an area micrometer. Although pressure and time-to-measurement standards were used that have been established previously [14] and subsequently used by others [5,22,23], the area measurement depends on these procedural variables. However since these procedural variables were standardized, the effects were systematic and hence did not affect the comparisons of cross-sectional area, stresses, and moduli since the comparisons depended on the differences in these properties.

4.2 Interpretation of Results. Since the purpose of this study was to determine whether the bovine tendon graft could be used as a substitute for a human tendon graft in in vitro experiments, the results should be interpreted in this context. One example of an in vitro experiment of particular importance is to determine the surgical variables (e.g., graft pretension, fixation method stiffness, tunnel placement) that provide the best match in anterior laxity between the reconstructed knee and the normal knee. To make this determination, each surgical variable could be varied systematically for a knee with an ACL reconstructed using a double-looped tendon graft and the laxity could be measured and compared to that for a normal knee. Among the graft properties that would potentially affect the laxity measurements would be the viscoelastic behavior and the tangent stiffness.

To interpret any differences in these properties determined in a uniaxial test, the allowable difference in knee laxity must be specified and then displacement differences in a uniaxial test must be translated into displacement differences in a laxity test. Considering that clinically a 3 mm side-to-side difference in laxity is indicative of an unstable knee, an acceptable systematic error in knee laxity would be 0.5 mm. For a graft that forms projection angles of 70 degrees with respect to the tibial plateau in both the coronal and saggital planes and spans the intra-articular distance of 30 mm, the graft must stretch along its axis 0.72 mm for an increase in anterior knee laxity of 0.5 mm to occur. Accordingly the knee laxity increases at a rate about 0.69 times that of the uniaxial stretch in the graft.

The viscoelastic behavior of the graft could affect in vitro knee laxity in two separate ways. One way is through the change in pretension that will occur as a result of load relaxation. The other way is through a change in elongation as the graft creeps while knee specimens are cyclically loaded for different knee conditions (e.g., different tunnel positions, different flexion angles, etc.). The constant displacement relaxation test followed by the constant amplitude load cyclic creep test was designed to investigate both of these ways. Inasmuch as the relaxation behaviors of the two grafts were virtually identical (Fig. 5), no appreciable error would be introduced into laxity. The constant amplitude load cyclic creep behaviors also demonstrated no significant differences (Fig. 6) and the difference in residual displacement at the end of the test was 0.03 mm and was likewise not significant.

The other graft property that would affect the laxity of a reconstructed knee subjected to an anterior load is the stiffness of the double-looped graft particularly in the toe region. The comparison between the bovine and human tendon grafts showed a significant difference in the tangent stiffness functions. The differences in the tangent stiffness functions between the two grafts translate into a displacement difference of 0.03 mm (Table 4) at a load of 250 N which was not significant statistically.

Another example of an in vitro experiment that would require the use of double-looped tendon grafts is the determination of the structural properties of femoral and tibial fixation methods. Structural properties of interest would be the fixation method stiffness since this affects the stiffness of the graft-fixation complex, the slippage, and the yield load. Both the slippage and yield load are quantities that describe the ability of the fixation method to support load without increasing the slackness of the graft and hence the laxity of a knee. One possible protocol for such a test would entail fixing the graft to either the femur or the tibia using the fixation method of interest, pulling along the axis of the tunnel with incrementally increasing tensile load, and then measuring the length change in the graft for each increment of the load [3,4]. All three structural properties can be computed from the resulting data. Thus the graft properties that would potentially affect the computations would be the viscoelastic behavior, the cross-sectional area, and the ultimate load.

The viscoelastic behavior would affect the computations because the slippage is a measure of the combined effects of the stretch of the tendon graft and the fixation slippage [3]. The incremental load cyclic creep test was designed to simulate the loads applied in slippage tests. Inasmuch as the residual creep displacements were virtually identical (0.04 mm difference) for the two graft materials (Table 3), the viscoelastic behaviors can be considered to be the same for the two grafts.

Because both the yield and slippage of most fixation devices are determined by the failure of the tendon-device interface rather than structural failure of the device itself [24–26], both the cross-sectional area and the ultimate load of the tendons will have a bearing on the device performance. Inasmuch as the cross-sectional areas differed on average by less than 3.5 mm^2 (Tables 4 and 5), it can be concluded that the areas are the same and hence this property is unlikely to cause any difference. Also the ultimate load of the bovine tendon graft was virtually identical to that of the human tendon graft (difference=13 N). Thus absolute measures of both the slippage and yield strength can be made with confidence using the bovine tendon grafts.

5 Conclusions

The conclusions from this study can be summarized as follows:

1 No statistically significant differences were evident between the viscoelastic behavior of the double–looped bovine and human tendon grafts rendering this property unimportant as a source of error in in vitro experiments aimed at measuring knee laxity. Also, although the tangent stiffness function of the bovine tendon grafts was different from that of the human tendon grafts, there was virtually no difference in displacement between the two grafts at 250 N of load so that any differences in tangent stiffness functions would not introduce appreciable error into laxity measurements. Inasmuch as the viscoelastic behavior and the stiffness in the toe region are the two most important properties relevant to substituting bovine grafts for human grafts in in vitro experiments aimed at the study of knee laxity, bovine grafts can be substituted for human grafts in these types of tests.

2 The creep in the incremental load creep tests, the cross-sectional area, and the ultimate load compared closely for both the bovine and human tendon grafts. Inasmuch as these are the most important graft properties relevant to substituting bovine grafts for human grafts in experiments aimed at evaluating fixation methods, bovine grafts can be substituted for human grafts in these types of tests.

Acknowledgments

The authors are grateful to the Whitaker Foundation for partial financial support of this study.

References

[1] Brown, C. H., Steiner, M. E., and Carson, E. W., 1993, ''The Use of Hamstring Tendons for Anterior Cruciate Ligament Reconstruction: Technique and Results,'' Clin. Sports Med., **12**, pp. 723–736.

[2] Howell, S. M., and Gottlieb, J. E., 1996, ''Endoscopic Fixation of a Double–Looped Semitendinosus and Gracilis ACL Graft Using a Bone Mulch Screw,'' Oper. Tech. Orthop., **6**, pp. 152–160.

[3] Liu, S. H., Kabo, J. M., and Osti, L., 1995, ''Biomechanics of Two Types of Bone-Tendon-Bone Grafts for ACL Reconstruction,'' J. Bone Jt. Surg., **77B**, pp. 232–235.

[4] Magen, H. E., Howell, S. M., and Hull, M. L., 1999, ''Structural Properties of Six Tibial Fixation Methods for Anterior Cruciate Ligament Soft Tissue Grafts,'' Am. J. Sports Med., **27**, pp. 35–43.

[5] To, J. T., Howell, S. M., and Hull, M. L., 1999, ''Contributions of Femoral Fixation Methods to the Stiffness of Anterior Cruciate Ligament Replacements at Implantation,'' J. Arth. Relat. Res., **15**, pp. 379–387.

[6] Eagar, P. J., Howell, S. M., and Hull, M. L., 1998, ''Effect of Fixation Method Stiffness and Graft Pretension on Restoring Normal Load-Displacement Behavior to the Knee With an ACL Reconstruction Using a Hamstrings Graft,'' ORS Trans., **24**, p. 939.

[7] Hamner, D. L., Brown, Jr., C. H., Steiner, M. E., Hecker, A. T., and Hayes, W. C., 1999, ''Hamstring Tendon Grafts for Reconstruction of the Anterior Cruciate Ligament: Biomechanical Evaluation of the Use of Multiple Strands and Tensioning Techniques,'' J. Bone Jt. Surg., **81-A**, pp. 549–557.

[8] Woo, S. L. Y., Gomez, M. A., and Akeson, W. H., 1981, ''The Time and History-Dependent Viscoelastic Properties of the Canine Medial Collateral Ligament,'' ASME J. Biomech. Eng., **103**, pp. 293–298.

[9] Kwan, M. K., Lin, T. H., and Woo, S. L. Y., 1993, ''On the Viscoelastic Properties of the Anterormedial Bundle of the Anterior Cruciate Ligament,'' J. Biomech., **26**, pp. 447–452.

[10] McMaster, W. C., 1986, ''Mechanical Properties and Early Clinical Experience With Xenograft Biomaterials,'' Bulletin of the Hospital for Joint Diseases Orthopedic Institute, **46**, pp. 174–184.

[11] Allen, P. R., Amis, A. A., Jones, M. M., and Heatley, F. W., 1987, ''Evaluation of Preserved Bovine Tendon Xenografts: A Histological, Biomechanical and Clinical Study,'' Biomaterials, **8**, pp. 146–152.

[12] Milthorpe, B. K., 1994, ''Xenografts for Tendon and Ligament Repair,'' Biomaterials, **15**, pp. 745–752.

[13] Ellis, D. G., 1969, ''Cross-Sectional Area Measurements of Tendon Specimens: a Comparison of Several Methods,'' J. Biomech., **2**, p. 175.

[14] Noyes, F. R., Butler, D. L., Grood, E. S., Zernicke, R. F., and Hefzy, M. S., 1984, ''Biomechanical Analysis of Human Ligament Grafts Used in Knee-Ligament Repairs and Reconstructions,'' J. Bone Jt. Surg., **66-A**, pp. 344–352.

[15] Goss, B. C., Hull, M. L., and Howell, S. M., 1997, ''Contact Pressure and Tension in Anterior Cruciate Ligament Grafts Subjected to Roof Impingement During Passive Knee Extension,'' J. Orthop. Res., **15**, pp. 263–268.

[16] Pioletti, D. P., Rakotomanana, L. R., and Leyvraz, P. F., 1999, ''Strain Rate Effect on the Mechanical Behavior of the Anterior Cruciate Ligament-Bone Complex,'' Med. Eng. Phys., **21**, pp. 95–100.

[17] Wallace, M. P., Howell, S. M., and Hull, M. L., 1997, ''In Vivo Tensile Behavior of a Four-Bundle Hamstring Graft as a Replacement for the Anterior Cruciate Ligament,'' J. Orthop. Res., **15**, pp. 539–545.

[18] Lam, T. C., Frank, C. B., and Shrive, N. G., 1992, ''Calibration Characteristics of a Video Dimension Analyser (VDA) System,'' J. Biomech., **25**, pp. 1227–1231.

[19] Lam, T. C., Shrive, N. G., and Frank, C. B., 1995, ''Variations in Rupture Site and Surface Strains at Failure in the Maturing Rabbit Medial Collateral Ligament,'' ASME J. Biomech. Eng., **117**, pp. 455–461.

[20] Graf, B. K., Vanderby, Jr., R., Ulm, M. J., Rogalski, R. P., and Thielke, R. J., 1994, ''Effect of Preconditioning on the Viscoelastic Response of Primate Patellar Tendon,'' Arthroscopy, **10**, pp. 90–96.

[21] Johnson, G. A., Tramaglini, D. M., Levine, R. E., Ohno, K., Choi, N. Y., and Woo, S. L. Y., 1994, ''Tensile and Viscoelastic Properties of Human Patellar Tendon,'' J. Orthop. Res., **12**, pp. 796–803.

[22] Smith, B. A., Livesay, G. A., and Woo, S. L. Y., 1993, ''Biology and Biomechanics of the Anterior Cruciate Ligament,'' Clin. Sports Med., **12**, pp. 637–670.

[23] Butler, D. L., Kay, M. D., and Stouffer, D. C., 1986, ''Comparison of Material Properties in Fascicle-Bone Units From Human Patellar Tendon and Knee Ligaments,'' J. Biomech., **19**, pp. 425–432.

[24] Good, L., Tarlow, S. D., Odensten, M., and Gillquist, J., 1990, ''Load Tolerance, Security, and Failure Modes of Fixation Devices for Synthetic Knee Ligaments,'' Clin. Orthop. Relat. Res., **253**, pp. 190–196.

[25] Steiner, M. E., Hecker, A. T., Brown, C. H. J., and Hayes, W. C., 1994, ''Anterior Cruciate Ligament Graft Fixation: Comparison of Hamstring and Patellar Tendon Grafts,'' Am. J. Sports Med., **22**, pp. 240–246.

[26] Robertson, D. B., Daniel, D. M., and Biden, E., 1986, ''Soft Tissue Fixation to Bone,'' Am. J. Sports Med., **14**, pp. 398–403.