Lengthening of a Single-Loop Tibialis Tendon Graft Construct After Cyclic Loading: A Study Using Roentgen Stereophotogrammetric Analysis

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Although single-loop tibialis tendon allografts have increased in popularity owing to their many advantages over patellar tendon and double-loop hamstring tendon autografts, some percentage of the patient population do not have clinically stable knees following anterior cruciate ligament reconstruction with single-loop tibialis tendon allografts. Therefore, it would be advantageous to determine the causes of increased anterior laxity which ultimately must be traced to lengthening of the graft construct. One objective of this study was to demonstrate the feasibility of using Roentgen stereophotogrammetric analysis (RSA) to determine the causes of lengthening of a single-loop graft construct subjected to cyclic loading. A second objective was to determine which cause(s) contributes most to an increase in length of this graft construct. Radio-opaque markers were inserted into ten grafts to measure the lengthening at the sites of the tibial and femoral fixations and between the sites of fixation. Each graft was passed through a tibial tunnel in a calf tibia, looped around a rigid cross-pin, and fixed to the tibia with a Washerloc fixation device. The grafts were cyclically loaded for 225,000 cycles from 20 to 170 N. Prior to and at intervals during the cyclic loading, simultaneous radiographs were taken. RSA was used to determine the three-dimensional coordinates of the markers from which the lengthening at the sites of fixation and between the sites of fixation was computed at each interval. The sites of the femoral and tibial fixations were the largest contributors to the increase in length of the graft construct, with maximum average values of 0.68 and 0.55 mm, respectively, after 225,000 cycles. The graft substance between the sites of fixation contributed least to lengthening of the graft, with a maximum average value of 0.31 mm. Ninety percent of the maximum average values occurred before 100,000 cycles of loading for the largest contributors. RSA proved to be a useful method for measuring lengthening due to all three causes. Lengthening of the graft construct at the sites of both fixations is sufficiently large that the combined contributions may manifest as a clinically important increase in anterior laxity.
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Introduction

Tibialis allografts have recently become more popular for anterior cruciate ligament (ACL) reconstructions due to their large cross section, increased stiffness and strength, ability to mimic the anatomy of the original ACL, reduced surgical time, and lack of donor site morbidity yet with similar outcomes as autografts [1–5]. Nevertheless clinically important increases in anterior knee laxity occur in 9–22% of knees during the first 2 years following ACL reconstruction with tibialis tendon allografts [1,3]. Although the cause(s) is unknown, increased anterior laxity can be traced to lengthening of the graft construct (i.e., graft-fixation-bone complex). Three possible causes of lengthening of the graft construct are lengthening at the sites of the tibial and femoral fixations [6–10] and lengthening between the sites of fixation (i.e., graft substance) [8–11]. To improve patient outcome, it is of interest to identify the contributions of the various causes to increases in anterior laxity which can be accomplished through a longitudinal in vivo study.

In a longitudinal in vivo study, increases in anterior laxity as well as the respective contributions of lengthening at the sites of fixation and between the sites of fixation must be measured over time in a patient cohort. Any method for measuring these contributions must be proven in an experimental cadaveric study before it can be confidently used in vivo. Roentgen stereophotogrammetric analysis (RSA) has been used previously to determine lengthening of a soft tissue graft [9,10,12], but neither study allowed for a complete analysis of lengthening. Therefore, the first objective of this study was to demonstrate the feasibility of using RSA to quantify lengthening of a single-loop graft construct by determining the lengthening at the sites of fixation and lengthening between the sites of fixation after cyclic loading. A related second objective was to determine which of the causes contributes most to lengthening of a single-loop graft construct.

The rehabilitation program following an ACL reconstruction is an important factor to the success of the procedure. Conservative rehabilitation programs, which restrict motion and passively exercise the knee, lead to muscle atrophy and delay return to normal activities [13]. Consequently, aggressive rehabilitation, which does not restrict motion and actively exercises the knee, is preferred to more quickly return the patient to normal activities [14]. Aggressive rehabilitation would be expected to cyclically load a

healing graft [15] during the period leading up to biological incorporation of the graft in the bone tunnels. During this period, fixation of the graft to bone relies solely on the fixation devices. Therefore, the final objective was to determine whether graft construct lengthening at the sites of fixation stabilizes during cyclic loading.

Materials and Methods

Specimen Preparation. To provide a tibial tunnel that simulated an ACL reconstruction, ten tibias were harvested from fresh-frozen calf knees and cemented in aluminum tubes. Calf tibias were used due to their availability, low cost, and because they have been used by previous studies as a model of young human tibias [6,9,16–18]. Each tibia was separated from its corresponding femur by sectioning all joining soft tissues. The distal end of each tibia was cut away and the remaining soft tissues were removed. Each tibia was cemented in an aluminum tube with polymethylmethacrylate so that the proximal end remained accessible for ACL reconstruction.

A tibial tunnel and counterbore were drilled in each tibia to place the ACL graft and fixation device. A one-step tibial guide (Arthrotek, Warsaw, IN) was set to a length of 45 mm and positioned with the bullet of the guide on the medial flare of the tibia at an angle of approximately 70 deg from the tibial plateau in the coronal plane. With the drill guide in this position, a guide wire was drilled through the tibia to orient the direction of the tunnel. Then, a 9 mm diameter cannulated reamer was used to bore out the tunnel. A 17 mm diameter counterbore was drilled perpendicular to the posterior wall of the tunnel at the distal opening until flush with the posterior wall of the tunnel.

Six radio-opaque markers were placed in the tibia to define a tibial coordinate system (Fig. 1). The markers (0.8 mm diameter tantalum balls, Tilly Medical Products AB, Lund, Sweden) were implanted in the tibia using a bead injector device (Tilly Medical Products AB, Lund, Sweden). Two markers ($T1$ and $T2$) were placed along the axis of the tibial tunnel through a specially designed guide tool [9]. The four remaining tantalum markers ($T3–T6$) were distributed in the proximal end of the tibia. Although only three markers are required to describe a coordinate system, the use of additional markers created an overdetermined system which reduced the error in determining the position of the tibia [19].

Bovine tendons were used to construct ten single-loop ACL grafts. Bovine tendons were used because the material and viscoelastic properties of single-loop grafts constructed from these tendons are similar to those of grafts constructed from human tibialis tendons used for reconstruction of the ACL [5,11]. For each graft, the middle extensor tendon was harvested from a bovine forelimb of a skeletally mature animal. The tendon was sized by assuring that the folded graft just passed through a 9 mm diameter sizing sleeve (Arthrotek, Warsaw, IN). Each end of the tendons was sewn with a No. 1 suture (U.S. Surgical, Norwalk, CT) using the whip stitch method. For each tendon, the free ends of the sutures were tied together forming a loop (tendon and suture). The tendons were immersed in saline and stored at −20°C.

Before the graft was fixed to the tibia, four markers were inserted in the graft ($G1–G4$, Fig. 1) to study the lengthening of an ACL graft construct. The graft was folded in half, and each limb was marked at 0.5 and 6.0 cm from the looped end of the graft. In one bundle of the graft 1.0 mm markers were injected using a 16 gauge needle while 0.8 mm markers were injected into the other bundle using the bead injector device. All markers were injected perpendicular to the long axis of the graft. Direct injection of markers into single-loop tibialis grafts introduces negligible error due to marker migration during cyclic loading [20].

Experiments. Each tibia, cemented in a metal cylinder, was clamped by a custom fixture in turn attached to the actuator of a materials testing machine (Table Top 858, MTS Corporation, Min-
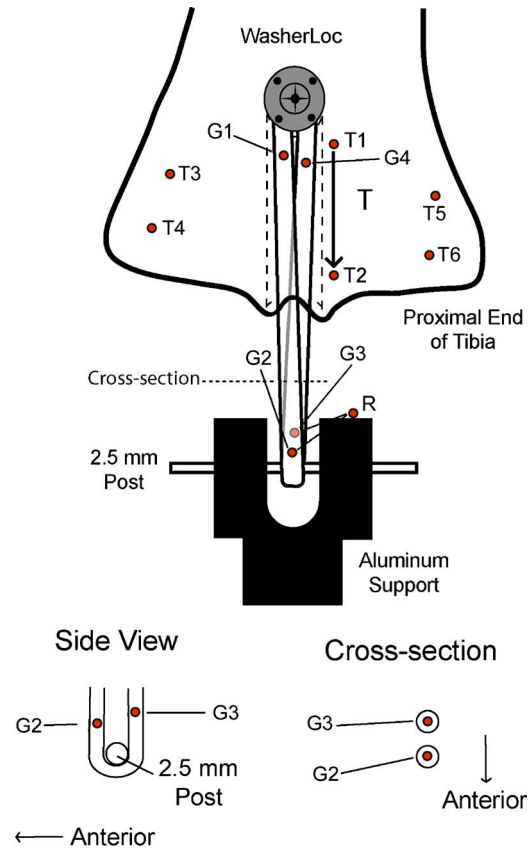

Fig. 1 Anterior view of the tibia showing the placement of tantalum markers in the bone ($T1–T6$), tendon markers in the graft ($G1$, $G2$, $G3$, $G4$), and a marker cemented to the support containing the rigid cross pin (R). The vector T is drawn to indicate the axis of the tibial tunnel. The axis of the tunnel coincides with the direction of loading.

neapolis, MN). The custom fixture aligned the tibial tunnel (and ACL graft) with the axis of loading. An aluminum support containing a 2.5 mm diameter steel cross pin was attached to the base of the machine. The cross pin was positioned in line with the tibial tunnel at a distance of 25–30 mm and standardized for a graft length of 75 mm from the distal opening. A tantalum marker (R) was cemented to the cross-pin support (Fig. 1). The graft was passed through the tibial tunnel, looped around the cross pin, and immersed in a saline bath (0.9% isotonic solution). The two bundles of the ACL graft were equally tensioned from the distal opening of the tibial tunnel using a custom jig and hanging weights of 10 N each [9]. An extended-spike WasherLoc and cancellous bone screw (Arthrotek, Warsaw, IN) were used to fix the ACL graft to the tibia. The WasherLoc was used because lengthening at the site of fixation is less using this device than other tibial fixation devices [7].

A calibration cage and two portable x-ray machines were used to perform RSA. The calibration cage (Tilly Medical Products AB, Lund Sweden) was made from plexiglass and contained markers at known positions to be used for system calibration. Modifications to the calibration cage were made to hold two x-ray cassettes and two scatter grids (Medical X-Ray Enterprises, Inc, Culver City, CA) placed at right angles to each other (AP and lateral views). The scatter grids were used to minimize exposure of the radiographic film from nonincident rays and thus improve image quality. The calibration cage surrounded the tibia and ACL graft so that all markers could be seen from each view. Each portable x-ray machine (MinXray Inc., Northbrook, IL) was positioned a distance of between 90 and 98 cm from its respective film

plane so that the direction of rays was orthogonal to this plane. The exposure of the x-ray machines was set to 80 kVp, 0.15 mA and the time was adjusted as needed depending on the bone density and thickness of the tibia.

Using the materials testing machine, each ACL graft was cyclically loaded to mimic the loading that the graft might experience during postoperative rehabilitation. The grafts were loaded for 225,000 cycles from 20 to 170 N at 10 Hz. The maximum load was equal to the maximum force that has been estimated to occur in the ACL during level walking [15]. The loading frequency was chosen to complete the test in a single day.

Simultaneous radiographs were taken before loading and at intervals during the test to study lengthening at the sites of fixation and between the sites of fixation as a function of time. Before cyclic loading, a tare load of 20 N was applied to the graft and radiographs were taken to record the initial position of the markers. Additional radiographs were taken during the test after 100, 225, 500, 1000, 2250, 5000, 10,000, 22,500, 50,000, 100,000, and 225,000 cycles. The intervals were chosen to represent the lengthening of the graft early as well as late in the rehabilitation. At each interval, the load on the ACL graft was returned to 20 N and held for 20 s to allow the load to equilibrate. Then the displacement of the actuator (and ACL graft) was held constant as the radiographs were taken.

Data Analysis. Analysis of the radiographs was performed using a custom RSA system which has been described previously [9] and will be summarized here. A digital image was obtained of each radiograph using a backlit scanner (Epson 1600, Epson America Inc., Long Beach, CA). The two-dimensional coordinates of all markers in each image were measured from the digital image using a software program (Scion Image 1.0, Scion Corporation, Frederick, MD).

A customized program written in MATLAB (version 5.3, The Mathworks Inc., Natick, MA) was used to determine lengthening at the sites of fixation and between the sites of fixation. This program computed the transformation of image coordinates to the calibration cage, the positions of the roentgen foci, and the three-dimensional position coordinates of all the markers. From the radiographs taken prior to cyclic loading, a tibial coordinate system was created using the first three tibia markers ($T1-T3$) such that the x-axis was defined by the axis of the tunnel (and direction of load). The first tibia marker ($T1$) was chosen as the tibial origin. A subroutine was written to transform the position coordinates of the markers from the laboratory coordinate system (defined by the calibration cage) to the tibial coordinate system. Because the position of the tibia (and tibial coordinate system) with respect to the calibration cage changed during the test, it was necessary to recompute the transformation from the laboratory coordinate system to the tibial coordinate system for each subsequent set of radiographs. For each set of radiographs, all marker positions were expressed in the tibial coordinate system. Using this custom RSA system, the error in determining the distance between two markers was 0.046 mm [9].

Lengthening at the site of the tibial fixation was determined using the two tendon markers placed closest to the WasherLoc ($G1$ and $G4$ in Fig. 1). From each set of radiographs, the position vectors $\mathbf{P}^{G1/T1}$ and $\mathbf{P}^{G4/T1}$ locating the tendon marker from the tibial origin were computed. The amount of relative motion between the graft and the tibia (T) was determined as the average vector change of $\mathbf{P}^{G1/T1}$ and $\mathbf{P}^{G4/T1}$ from the initial vector, projected along the axis of the tibial tunnel by the equation

$$T = \frac{\Delta \mathbf{P}^{G1/T1} \cdot \mathbf{T} + \Delta \mathbf{P}^{G4/T1} \cdot \mathbf{T}}{2|\mathbf{T}|} \qquad (1)$$

where $\Delta \mathbf{P}^{G1/T1}$ and $\Delta \mathbf{P}^{G4/T1}$ are the vector differences (final minus initial) of the vector to $G1$ from $T1$ and the vector to $G4$ from $T1$, respectively, while $\mathbf{T}$ is the vector along the axis of the tibial

tunnel.

The amount of lengthening between fixations was determined using the two tendon markers in the same tendon bundles ($G1$ and $G2$, $G4$ and $G3$ in Fig. 1). From each set of radiographs, the vectors $\mathbf{P}^{G2/G1}$ and $\mathbf{P}^{G3/G4}$ connecting the two tendon markers were computed. The amount of lengthening between fixations (B) was determined as the average vector change of $\mathbf{P}^{G2/G1}$ and $\mathbf{P}^{G3/G4}$ from the initial vector, projected along the axis of the tibial tunnel by the equation

$$B = \frac{\Delta \mathbf{P}^{G2/G1} \cdot \mathbf{T} + \Delta \mathbf{P}^{G3/G4} \cdot \mathbf{T}}{2|\mathbf{T}|} \qquad (2)$$

where $\Delta \mathbf{P}^{G2/G1}$ and $\Delta \mathbf{P}^{G3/G4}$ are the vector differences (final minus initial) of the vector to $G2$ from $G1$ and to $G3$ from $G4$, respectively.

The amount of lengthening at the site of the femoral fixation was determined using the tendon markers placed nearest to the rigid cross pin ($G2$ and $G3$ in Fig. 1). Because the graft was looped around a rigid cross pin, the lengthening at this site was due only to local contact deformation as a result of compression of the graft around the rigid cross pin. From each set of radiographs, the vectors $\mathbf{P}^{G2/R}$ and $\mathbf{P}^{G3/R}$ to the tendon markers placed near the cross pin from the marker cemented to the support containing the rigid cross pin were computed. Lengthening at the site of the femoral fixation (F) was determined as the average vector change of $\mathbf{P}^{G2/R}$ and $\mathbf{P}^{G3/R}$ from the initial vector, projected along the axis of the tibial tunnel by the equation

$$F = \frac{\Delta \mathbf{P}^{G2/R} \cdot \mathbf{T} + \Delta \mathbf{P}^{G3/R} \cdot \mathbf{T}}{2|\mathbf{T}|} \qquad (3)$$

where $\Delta \mathbf{P}^{G2/R}$ and $\Delta \mathbf{P}^{G3/R}$ are the vector differences (final minus initial) of the vector to $G2$ from R and to $G3$ from R, respectively.

The absolute contributions of lengthening at the sites of fixation and between the sites of fixation to the increase in length of the graft construct were determined at every interval. Using the data from all ten specimens, the average value of each variable was computed and plotted according to cycle interval.

To determine the primary cause(s) of graft lengthening, the data was analyzed using a repeated measures analysis of variance (ANOVA). As an initial analysis, a two-factor repeated measures ANOVA was used in which the two factors were the cause of graft lengthening at three levels (site of tibial fixation, site of femoral fixation, and between the sites of fixation) and the number of cycles at eleven levels (100, 225, 500, 1000, 2250, 5000, 10,000, 22,500, 50,000, 100,000, and 225,000 cycles). Because the results from the two-factor ANOVA revealed a significant interaction between the two factors ($p=0.0368$), a series of eleven single-factor ANOVAs were performed at each cycle interval. A Tukey's test with the level of significance $\alpha=0.05$ was used to determine which cause(s) were significantly different at each cycle interval.

Results

Lengthening at the sites of the fixations were the largest contributors to the increase in length of the graft construct while lengthening between the sites of fixation contributed the least (Fig. 2). Lengthening at the sites of fixation was significantly greater than lengthening between the sites of fixation above 100 cycles ($p<0.05$ from Tukey) but were not significantly different from one another ($p>0.05$ from Tukey) (Table 1). Lengthening at the sites of fixation increased to maximum average values of 0.552 mm for the tibial fixation and 0.679 mm for the femoral fixation after 225,000 cycles (Table 1). Lengthening between the sites of fixation showed no obvious trend over the number of cycles but reached a maximum average value of 0.306 mm after 225,000 cycles.

Although lengthening at the sites of fixation and between the sites of fixation generally increased throughout the test, the change in each variable decreased as the number of cycles in-
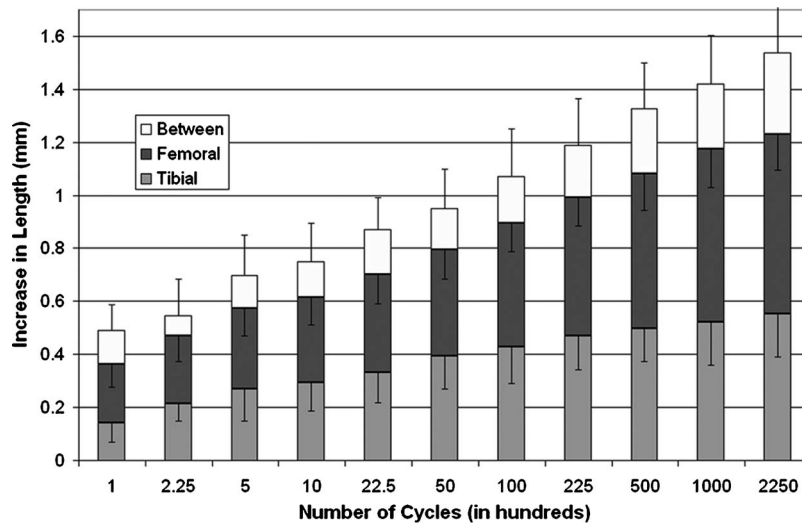
Fig. 2 Contributions of lengthening at the sites of the fixations and between the sites of fixation to the total increase in length of single-loop tendon graft constructs after cyclic loading ($n=10$). The error bars indicate one standard deviation from the mean.

creased (Fig. 3). At the sites of tibial and femoral fixation, 90% and 86% of the maximum lengthening respectively occurred at 50,000 cycles. Between the sites of fixation, 80% of the maximum lengthening occurred at 100,000 cycles.

Discussion

Although single-loop tibialis tendon allografts have increased in popularity owing to their many advantages over patellar tendon and double-looped hamstring tendon autografts, some percentage of the patient population with knees reconstructed with single-loop tibialis tendon allografts do not benefit from a clinically stable knee. Therefore, it would be advantageous to determine the causes of increased anterior laxity which ultimately must be traced to lengthening of the graft construct. Because lengthening between the sites of fixation is one cause of lengthening of the graft construct and may occur due to graft remodeling in the biological environment, any study aimed at determining the causes of increased anterior laxity should be performed on a patient cohort. Thus one objective of the work reported in this paper was to demonstrate the feasibility of using RSA to quantify lengthening of a single-loop graft construct in a longitudinal in vivo study. A second objective was to determine the respective contributions from each of the three causes while a final objective was to determine whether lengthening at the two sites of fixation stabilizes during cyclic loading. One key finding was that RSA is an effective method for measuring lengthening of the graft construct at the sites of tibial and femoral fixation and between the sites of fixation. A related key finding was that the lengthening at the sites of fixation contributes more to the lengthening of the graft construct than lengthening between the sites of fixation. A final key finding

was that at least 86% of the lengthening occurred by 50,000 cycles for both the tibial and femoral fixations.

Various methodological issues associated with the test method used in this study were discussed previously in detail [9] so that a summary will be presented here. In this discussion it was concluded that the two error sources (i.e., inherent error of the RSA method=0.046 mm [9] and migration of markers=0.100 mm [20]) did not affect our ability to measure the amounts of lengthening in this study because the errors were relatively small and random in nature. Also the amplitude, frequency, and number of load cycles were justified. Next lengthening between the sites of fixation and lengthening at the sites of fixation were overestimated and underestimated respectively due to the small (i.e., 5 mm) distance separating the markers from the fixations but these errors were negligible. The amount of lengthening between the sites of fixation measured in this study is not necessarily representative of the amount that would occur in vivo because of the effects of graft remodeling in the biological environment. Finally, the femoral fixation was modeled after cross-pin femoral fixation devices which are in common use clinically and both the diameter and length of the cross pin used were the same as those of a commercially available device (Bone Mulch Screw, Arthrotek, Warsaw, IN).

Although RSA was demonstrated to be an effective method for measuring lengthening at the sites of fixation and lengthening between the sites of fixation in this study, several adaptations of the method used herein would need to be made in an in vivo study. Measuring lengthening at the sites of fixation is complicated by the fact that relative motion of two separate types can occur. One type is any relative motion between the fixation device and the

Table 1 Average lengthening at the site of each of the fixations, between the sites of fixation, and the total of all three after cyclic loading. The p-value obtained from each single-factor ANOVA and the results from Tukey's test are also given. Values indicated with an asterisk (*) are not significantly different ($\alpha=0.05$).

|	Number of Cycles										
	100	225	500	1000	2250	5000	10000	22500	50000	100000	225000
Femoral (mm)	0.221*	0.254*	0.304*	0.324*	0.370*	0.400*	0.464*	0.520*	0.585*	0.654*	0.679*
Tibial (mm)	0.142*	0.214*	0.269*	0.292*	0.331*	0.395*	0.430*	0.471*	0.497*	0.521*	0.552*
Between (mm)	0.127*	0.079	0.127	0.132	0.170	0.156	0.177	0.197	0.244	0.245	0.306
Total Contribution (mm)	0.490	0.548	0.700	0.749	0.871	0.951	1.071	1.188	1.326	1.420	1.538
ANOVA p-value	0.0490	0.0020	0.0094	0.0027	0.0014	0.0002	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
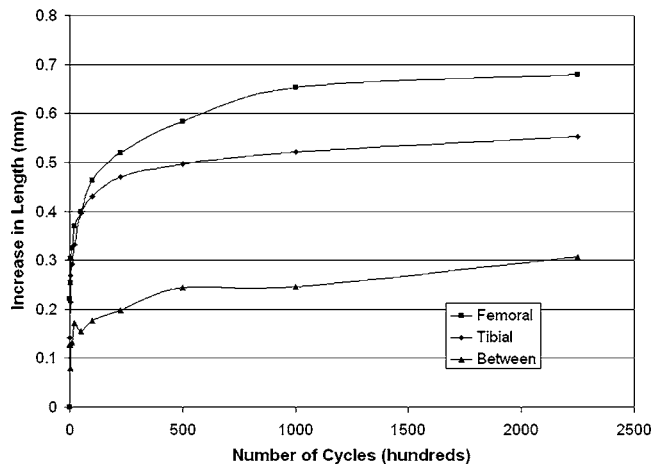
Fig. 3 Average lengthening at the sites of the fixation and between the sites of fixation in single-looped tendon graft constructs after cyclic loading. The horizontal axis is a linear scale. Lengthening at the sites of fixation reached approximately 86% of the final value by 50,000 cycles while lengthening between the sites of fixation reach 80% of the final value after 100,000 cycles.

bone and the other type is any relative motion between the graft and fixation device. Both types are important to determine because the dominance of one type over another would indicate that different remedial measures are necessary to prevent that type of relative motion.

In this study, the relative motion between the graft and fixation device was measured for the femoral fixation. The femoral fixation device consisted of a rigid cross pin anchored to the base of the materials testing system so that any relative motion between the fixation device and materials testing system was negligible and was not measured. In the in vivo application, the method easily could be adapted to measure the relative motion between the femoral fixation device and bone by attaching a marker to the femoral fixation device and also by implanting another arrangement of six markers in the femur similar to the arrangement of six markers implanted in the tibia. With the six markers implanted in the femur, a local femur-fixed coordinate system can be established [21] thus enabling any relative motion between the femoral fixation device and the femur to be determined.

Even though the graft was attached to the tibia with a tibial fixation device (i.e., Washerloc), the total of the two relative motions was measured rather the separate relative motions. Because a marker was not placed on the tibial fixation device, the possibility to determine any relative motion between the tibial fixation device and the tibia was precluded. However, both relative motions readily could be determined in the in vivo application by simply attaching a marker to the tibial fixation device.

Although lengthening between the sites of fixation was measured directly in this study using RSA, making a similar measurement in an in vivo study may be impractical for two reasons. Although this study was designed to determine the lengthening between the sites of fixation by pulling the graft in a straight line, measuring this quantity in vivo is more difficult because the graft does not typically follow a straight path. Moreover, because lengthening between the sites of fixation could occur beyond the time required for biological incorporation of the graft in the bone tunnels, determining lengthening between the sites of fixation in vivo would require that markers be attached to the graft in the intraarticular space. This would present the attendant risk that the markers may detach from the graft over time in which case they could become foreign bodies in the joint. Accordingly these two considerations may render the technique impractical for determining lengthening of the graft substance in the intra-articular space.

Notwithstanding the impracticality of measuring lengthening between the sites of fixation, this quantity can still be determined by adapting the RSA techniques used in this study. In this adaptation, the increase in anterior knee laxity would be measured using previously described techniques [21] and the lengthening in the regions of fixation would be measured using the techniques described in this study between two time points. Because a one-to-one relationship exists between lengthening of the graft construct and an increase in anterior laxity [8], the anterior laxity increase attributable to lengthening at both sites of fixation can be computed between these two time points. Any remainder in the anterior laxity increase necessarily would be attributable to lengthening between the sites of fixation and the amount of lengthening in between the sites of fixation would equal this remainder. Thus through simple adaptations of the techniques described in this study, all important causes of lengthening of the graft construct in vivo can be determined.

Of the three amounts of lengthening determined in this study, lengthening at the site of the femoral fixation was the largest at 0.68 mm and hence contributed the most to lengthening of the graft construct. This may seem surprising that lengthening at the site of the femoral fixation was greater than that at the site of the tibial fixation (0.55 mm) because the total lengthening (i.e., from both relative motions) was measured for the tibial fixation whereas only the relative motion between the graft and fixation device was measured for the femoral fixation. However, the mechanism of lengthening at the site of the cross-pin femoral fixation is due to contact deformation as water is squeezed out of the graft as it wraps around the cross pin [9,10]. For a single-loop graft such as that used in this study with a relatively large cross-sectional area, the results of this study indicate that the contact deformation is substantial. Lengthening at both sites of fixation reached 86 percent of their maximum values at 50,000 cycles which is well before biological incorporation of the graft would be expected to occur [22]. Thus their combined effect would contribute to an increase in anterior laxity of 1.3 mm on average.

Not surprisingly, lengthening of the graft between the sites of fixation was the smallest of the three contributions. For this in vitro study, lengthening between the sites of fixation was due to creep as a result of the cyclic loading. The average value of 0.31 mm compares favorably to the 0.3−0.4 mm range of creep previously reported for a constant load creep test of single-loop anterior and posterior tibialis tendon grafts [5]. However, creep under the cyclic loading applied in this study took approximately 100,000 cycles to reach 80% of the steady-state value in contrast to the constant load creep test which required only 15 min to reach steady state. This result demonstrates the high number of load cycles required in a cyclic load creep test to reach steady state [9,10].

In summary, our study has demonstrated the feasibility of using RSA to determine lengthening of single-loop graft constructs at the sites of fixation and between the sites of fixation after 225,000 cycles of load. Lengthening at the sites of fixation contributed the most to the lengthening of the graft construct, with maximum average values of 0.68 and 0.55 mm for the femoral and tibial fixations respectively after 225,000 cycles. Lengthening between the sites of fixation contributed least to lengthening of the graft construct in this in vitro study, with a maximum average value of 0.31 mm. Lengthening at both sites of fixation stabilized well before biological incorporation of the graft occurs so that their combined effect would manifest as an increase in anterior knee laxity.

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