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Lengthening of double-looped tendon graft constructs in three regions after cyclic loading: a study using Roentgen stereophotogrammetric analysis

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Abstract

Lengthening of a double-looped tendon graft construct used to reconstruct the anterior cruciate ligament (ACL) can result in an increase in anterior knee laxity and affect the stability of the reconstructed knee. Three possible regions where lengthening of the construct can occur are (1) the region of the tibial fixation, (2) the region of the femoral fixation, and (3) the region of the graft between the fixations. One objective of this study was to demonstrate the feasibility of using Roentgen stereophotogrammetric analysis (RSA) to determine the lengthening in each region of a double-looped graft construct subjected to cyclic loading. A second objective was to determine which region(s) contributes most to an increase in length of this graft construct. Radio-opaque markers were attached to ten grafts to measure the lengthening in each of the three regions. Each graft was passed through a tibial tunnel in a bovine 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 of the tibia and graft. RSA was used to determine the 3-dimensional coordinates of the markers from which the lengthening in each region was computed at each interval. The regions of the tibial and femoral fixations were the largest contributors to the increase in length of the graft, with maximum average values of 0.91 and 0.76 mm respectively after 225,000 cycles. The region between the fixations contributed least to lengthening of the graft, with a maximum average value of 0.23 mm. More than 90% of the lengthening in each region occurred before 100,000 cycles of loading. RSA proved to be a useful method for measuring lengthening in all three regions of the graft construct. Lengthening of the graft construct in both regions of fixation is sufficiently large that the combined contributions may cause a recurrence of instability in some knees.

Introduction

Previous research has shown that a clinically important increase in anterior knee laxity occurs in 10% to 20% of knees during the first 4 months following reconstruction of the anterior cruciate ligament (ACL) with double-looped tendon grafts [10,11]. Although the exact cause for the increase is unknown, one mechanism that results in an increase in anterior knee laxity is lengthening of the ACL graft construct [4].

Three possible regions in which lengthening of a double-looped tendon graft construct can occur are (1) the region of the tibial fixation, (2) the region of the femoral fixation, and (3) the region of the graft between the fixations. Lengthening of the graft construct in the regions of fixation has been observed in vitro in various amounts depending on the fixation devices used and the magnitude of load applied [7,9,12]. Creep has been demonstrated in cyclic loading of double-looped tendon grafts [8] and would cause lengthening in the region between the fixations.

Though lengthening of a double-looped tendon graft construct can occur in three regions, the respective contributions have not been studied. Knowledge of the amount and timing of lengthening in each region postoperatively could be used to reduce the incidence of knee instability following reconstructive surgery. Therefore, it would be beneficial to develop a method that could be used to determine the amount of lengthening that occurs in each region postoperatively.

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One method that has been used to study 3-dimensional relative motions between bones in vivo is Roentgen stereophotogrammetric analysis (RSA). This method uses simultaneous biplanar radiography to image radio-opaque markers (usually made of tantalum) implanted in tissues and reconstructs their 3-dimensional position coordinates. The advantage of using RSA to study lengthening in each of the three regions of a graft construct is the high accuracy with which changes in relative motions can be determined. The error of RSA has been determined to be about 0.05 mm for the translation of rigid bodies [19]. RSA has been used to study lengthening in the regions of fixation in bone-patellar tendon-bone grafts where the markers were placed in the bone plugs [5]. However, RSA has not been applied to study lengthening of soft tissue graft constructs. Therefore, the first objective of this study was to demonstrate the feasibility of using RSA to determine lengthening in each of the three regions of a double-looped soft tissue graft construct.

Rehabilitation after reconstruction of the ACL is an important factor in the outcome of the procedure. Aggressive rehabilitation is preferred to reduce muscle atrophy and to quickly return patients to normal activities [1]. Aggressive rehabilitation, which involves early motion and weight bearing activities such as walking, 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. Because cyclic loading of both the graft [8] and the fixations [7,9,12] causes lengthening in all three regions, knowledge of the primary region that results in graft lengthening could lead to methods for minimizing the lengthening in this region hence reducing the increase in anterior knee laxity that occurs post-operatively. Therefore, the second objective of this study was to identify which of the three regions contributes most to an increase in length of the graft construct.

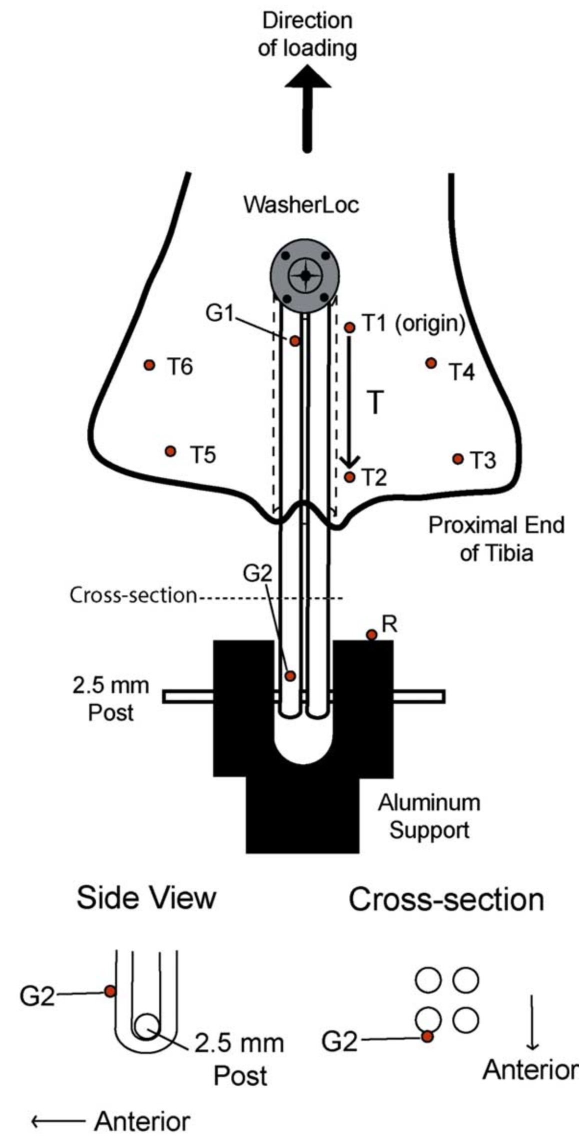

Fig. 1. Anterior view of the tibia showing the placement of tantalum markers in the bone (T1–T6), tendon markers in the graft (G1 and G2), and a marker cemented to the support containing the rigid cross-pin (*R*). The vector **T** is drawn to indicate the axis of the tibial tunnel. The axis of the tunnel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

Materials and methods

Specimen preparation

To provide a tibial tunnel that simulated an ACL reconstruction, ten tibias were harvested from fresh-frozen mature bovine knees ranging in age from 18 to 24 months. Bovine tibias were used due to their availability, low cost, and previous use as a model of young human tibias [3,7]. 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 (PMMA) 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° 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 21-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, Biomet Orthopedics Inc., Warsaw, IN) 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 [18]. The markers were placed at an equal depth so that the line connecting them was parallel to the axis of the tunnel. 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 and reduced the error in determining the position of the segment [22].

Bovine tendons were used to construct ten double-looped ACL grafts approximately 95 mm in length. Bovine tendons were used because the material and viscoelastic properties of double-looped grafts constructed from these tendons are similar to those of grafts constructed from human tendons used for reconstruction of the ACL [8]. For each graft, the middle extensor tendon was harvested from a bovine forelimb of a skeletally mature animal. The naturally bifurcated tendon was divided in two halves and trimmed so that the folded double-looped graft just passed through a 9-mm diameter sizing sleeve (Arthrotek, Warsaw, IN). Each end of both tendons was sewn with a #1 suture (US 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 being fixed to the tibia, the ACL graft was positioned in the tibial tunnel and equally tensioned. The graft was passed through the tunnel and looped around a cross-pin positioned proximal to the tibial plateau. The cross-pin was positioned in line with the tunnel at a distance of 50 mm from the proximal opening. The four bundles of the ACL graft were equally tensioned from the distal opening of the tunnel using a custom jig and hanging weights [8]. A 1.0 kg weight was suspended from each tendon, thereby applying a 20 N tensile load to the ACL graft. With the 20 N load applied, the edge of the distal opening of the tunnel was marked on the ACL graft with an ink marker. The weights were then removed and the ACL graft was partially pulled out of the tunnel so that the tendon markers could be attached.

To study the lengthening of a double-looped ACL graft construct in the three regions, two tendon markers were attached to one tendon bundle of each graft. The methods for the construction and attachment of the tendon markers are described in detail elsewhere [18]. In short, each tendon marker was constructed from a 0.8 mm tantalum ball and a stainless steel suture. The suture was wrapped around the ball so as to form a wire cage (Fig. 2). The tendon markers were sewn to the tendon bundle using the needle ends of the stainless steel suture. The first tendon marker (G1) was attached to the tendon bundle 5 mm from the tibial fixation device at the inner part of the four-bundle graft (Fig. 1). A second tendon marker (G2) was attached to the tendon bundle 5 mm from the rigid cross-pin. Once the tendon markers were sewn to the tendon, the weights were reapplied to tension the graft.

With the strands of the graft equally tensioned, the tibial fixation device was secured. A Washerloc spiked washer and cancellous bone screw (Arthrotek, Warsaw, IN) were used to fix the ACL graft to the tibia. The Washerloc was used because the lengthening in the region of fixation is less using this device than other tibial fixation devices [12].

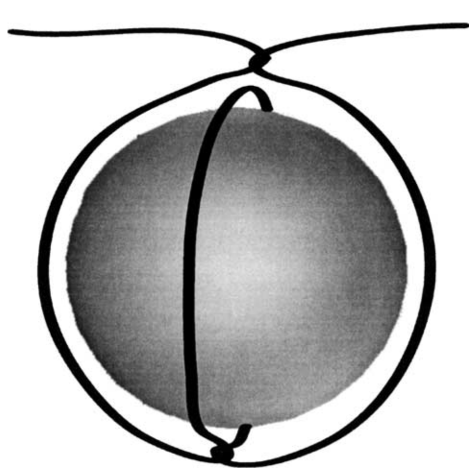

Fig. 2. Diagram of completed tendon marker composed of a 0.8-mm diameter tantalum ball and stainless steel suture. The tantalum ball is contained in a basket, with four arms along the sides, made from the stainless steel suture.

Experiments

Cyclic loading of the ACL grafts was applied by a materials testing machine (Table Top 858, MTS Corporation, Minneapolis, MN). Each tibia, cemented in a metal cylinder, was clamped to the cross-head of the machine using a custom fixture that aligned the tibial tunnel (and ACL graft) with the axis of loading. An aluminum support containing a 2.5-mm diameter cross-pin was attached to the base of the machine. A tantalum marker (R) was cemented to the support (Fig. 1). Once the tibia was secured to the cross-head and the tibial tunnel aligned, the graft was looped around the cross-pin and immersed in a saline bath (0.9% isotonic solution) at room temperature.

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 non-incident 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 85 and 110 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 3 mAs and adjusted as needed depending on the bone density and thickness of the tibia.

Each ACL graft was cyclically loaded to mimic the magnitude of loading and number of load cycles that the graft might experience during the early healing period before biological incorporation of the graft in the bone tunnels. The grafts were loaded for 225,000 cycles from 20 to 170 N at 8 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 in each of the three regions as a function of cycle number. Before cyclic loading, a tensile load of 20 N was applied to the graft and radiographs were taken to record the initial positions 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 that occurs early as well as later in the rehabilitation. At each interval, the load on the ACL graft was returned to 20 N and held for 20 seconds to allow the load to equilibrate. Then the displacement of the cross-head (and ACL graft) was held constant as the radiographs were taken.

Data analysis

Analysis of the radiographs was performed using a customized RSA system. A digital image was obtained of each radiograph using a back-lit scanner (Epson 1600, Epson America Inc., Long Beach, CA). The radiographs were scanned at a resolution of 300 dpi. The appearance of the digital images was modified using contrast and threshold controls from image editing software (Photoshop 5.0, Adobe Systems Inc., San Jose, CA) to optimize the identification of the markers in the image. The 2-dimensional coordinates of all markers were measured from each 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 in each of the three regions. This program computed the transformation of image coordinates to the calibration cage, the positions of the Roentgen foci, and the 3-dimensional position coordinates of all the markers [19]. 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. A Nelder–Mead simplex method was used to compute the transformation from the laboratory coordinate system to the tibial coordinate system by determining the set of matrix parameters that minimized the sum of squared distances of the transformed coordinates of the tibia markers from their known position in the tibial coordinate system determined from the initial set of radiographs. For each set of radiographs, all marker positions were expressed in the tibial coordinate system. Using our RSA system hardware and software, the root mean squared error in the distance between two markers is 0.046 mm [18].

The lengthening in the region of the tibial fixation was determined using the tendon marker placed closest to the Washerloc (G1 in Fig. 1). From each set of radiographs, the position vector $\mathbf{P}^{G1/T1}$ locating the tendon marker from the tibial origin was computed. Lengthening in the region of the tibial fixation ΔL_T was determined as the vector change of $\mathbf{P}^{G1/T1}$ from the initial vector projected along the axis of the tibial tunnel by the equation

$$\Delta L_T = \Delta\mathbf{P}^{G1/T1} \bullet \mathbf{T} \tag{1}$$

where $\Delta\mathbf{P}^{G1/T1}$ is the vector difference (final minus initial) of the vector to G1 from T1 and $\mathbf{T}$ is the vector along the axis of the tibial tunnel.

The lengthening in the region of the graft construct between the fixations was determined using the two tendon markers in the tendon bundle (G1 and G2 in Fig. 1). From each set of radiographs, the vector $\mathbf{P}^{G2/G1}$ connecting the two tendon markers was computed. Lengthening in the region of the graft construct between the fixations ΔL_G was determined as the vector change of $\mathbf{P}^{G2/G1}$ from the initial vector projected along the axis of the tibial tunnel by the equation

$$\Delta L_G = \Delta\mathbf{P}^{G2/G1} \bullet \mathbf{T} \tag{2}$$

where $\Delta\mathbf{P}^{G2/G1}$ is the vector difference (final minus initial) of the vector to G2 from G1.

The lengthening in the region of the femoral fixation was determined using the tendon marker placed nearest to the rigid cross-pin (G2 in Fig. 1). From each set of radiographs, the vector $\mathbf{P}^{G2/R}$ to the tendon marker placed near the cross-pin from the marker cemented to the support containing the cross-pin was computed. Lengthening in the region of the femoral fixation ΔL_F therefore was determined as the vector change of $\mathbf{P}^{G2/R}$ projected along the axis of the tibial tunnel by the equation

$$\Delta L_F = \Delta\mathbf{P}^{G2/R} \bullet \mathbf{T} \tag{3}$$

where $\Delta\mathbf{P}^{G2/R}$ is the vector difference (final minus initial) of the vector to G2 from R.

The lengthening in each of the three regions was 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 region(s) of graft lengthening, the data were analyzed using a repeated measures analysis of variance (ANOVA). As an initial analysis, a two-factor repeated measures ANOVA was used in which one factor was the region of graft lengthening at three levels (region of tibial fixation, region between fixations, and region of femoral fixation) and the other factor was 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 ($p < 0.001$), 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 region(s) were significantly different at each cycle interval.

Results

Lengthening in both regions of fixation was considerably larger than lengthening in the region between the fixations (Fig. 3). Lengthening in each region of fixation was significantly greater than lengthening in the region between the fixations above 2250 cycles ($p < 0.05$ from Tukey's) but lengthening in each region of fixation was not significantly different from one another ($p > 0.05$ from Tukey's) (Table 1). Lengthening in the regions of the tibial and femoral fixations increased to maximum average values of 0.91 and 0.76 mm respectively after 225,000 cycles (Table 1) whereas lengthening in the region between the fixations reached a much smaller

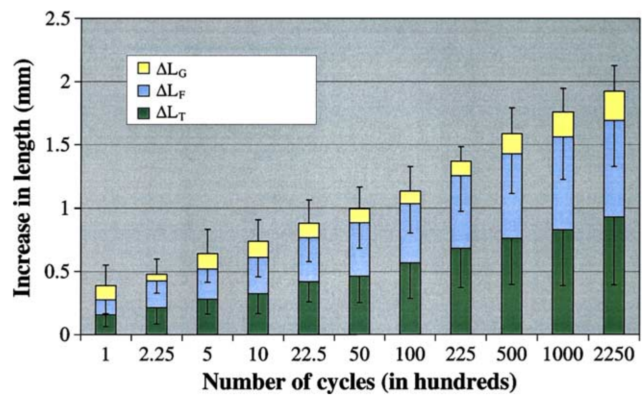

Fig. 3. Contributions in each of three regions to the increase in length of double-looped tendon graft constructs after cyclic loading ($n = 10$). ΔL_G is the lengthening in the region between the fixations, ΔL_F is the lengthening in the region of the femoral fixation, and ΔL_T is the lengthening in the region of the tibial fixation. The horizontal axis is a logarithmic scale. The error bars indicate one standard deviation from the mean.

Table 1
Average lengthening in the region of the tibial fixation ΔL_T, the region of the femoral fixation ΔL_F, and the region between the fixations ΔL_G and the total lengthening in all three regions (i.e. $\Delta L_T + \Delta L_F + \Delta L_G$) after cyclic loading

	Number of cycles										
	100	225	500	1000	2250	5000	10,000	22,500	50,000	100,000	225,000
ΔL_T (mm)	0.13*	0.20*	0.25*	0.31*	0.43*	0.46*	0.57*	0.68*	0.75*	0.82*	0.91*
ΔL_F (mm)	0.12*	0.21*	0.24*	0.29*+	0.35*	0.42*	0.46*	0.57*	0.66*	0.73*	0.76*
ΔL_G (mm)	0.11*	0.05+	0.12*	0.12+	0.12+	0.11+	0.10+	0.11+	0.16+	0.20+	0.23+
Total lengthening (mm)	0.36	0.46	0.61	0.72	0.89	0.99	1.14	1.36	1.58	1.75	1.90
ANOVA p-value	0.936	0.008	0.095	0.031	0.002	0.001	0.001	<0.001	<0.001	0.001	0.002

The p-value obtained from each single-factor ANOVA and the results from Tukey's test are also given. Values indicated with different symbols are significantly different ($\alpha = 0.05$).

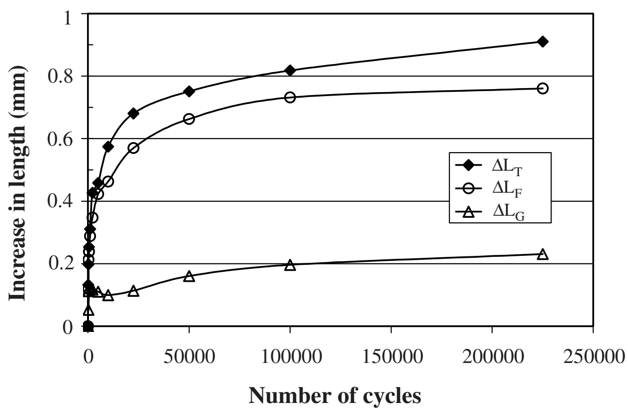

Fig. 4. Average increases in length in each of three regions in double-looped tendon graft constructs after cyclic loading. The horizontal axis is a linear scale. Ninety percent of the lengthening in the region between the fixations ΔL_G occurred by 50,000 cycles while 90% of the lengthening in the regions of the femoral fixation ΔL_F and tibial fixation ΔL_T occurred by 100,000 cycles.

maximum average value of 0.23 mm after 225,000 cycles.

Although lengthening in all three regions generally increased throughout the test, the change in each variable decreased as the number of cycles increased (Fig. 4). For the two regions of fixation, more than 90% of the maximum lengthening in each region occurred in fewer than 100,000 cycles. For the region between the fixations, more than 90% of the maximum lengthening occurred in fewer than 50,000 cycles.

Discussion

Because anterior knee laxity increases in approximately 10% to 20% of knees reconstructed with a double-looped tendon graft and because lengthening of the graft construct is the most likely mechanism responsible for the laxity increase, one objective of this study was to demonstrate the feasibility of using RSA to determine contributions to lengthening of a double-looped graft construct in each of three regions. A second objective was to identify which region contributed the most lengthening. The key results were that (1) RSA was demonstrated to be a useful technique for determining lengthening of the graft construct in each of three regions, (2) lengthening in the regions of fixation was substantially greater than lengthening between the fixations for this in vitro study, and (3) more than 90% of the lengthening in all three regions occurred within 100,000 cycles. Before discussing the importance of these findings, several methodological issues should be reviewed.

Methodological issues

Because lengthening of the graft construct in each of three regions was determined from the change in position of a single tendon marker (or change in relative position between two markers), there were two sources of error in determining the quantities of interest. One source was the inherent error in the RSA system and the other source was any migration of tendon markers that may have occurred as a consequence of repetitive loading. Of these two sources, migration contributes the greater amount of error, which has been estimated to reach 0.2 mm at 225,000 cycles [18]. Inasmuch as the error due to migration is random in nature, this error increased the variability in the computed quantities. Notwithstanding this increase in variability, the lengthening in both regions of fixation was significantly greater than that in the region between the fixations at intervals beginning after 2250 cycles and beyond. Consequently the added variability did not inhibit the ability to detect statistically significant differences with the ten specimens used in this study.

To minimize the number of tendon markers attached to the grafts, one bundle of a single tendon was marked to determine the quantities of interest. Although these quantities may not be the same for each bundle in a particular specimen, it is expected that the average values obtained in this study are representative of all four bundles because the marked bundle was varied randomly between specimens. Additionally, the lengthening of either tendon construct must be equal to the lengthening of the graft construct because the graft construct was always held in tension.

The lengthening in the region between the fixations was underestimated while the lengthening in both regions of fixation was overestimated to some degree. Ideally the tendon markers would have been placed immediately adjacent to the fixations but some distance between the markers and the fixations was necessary to prevent the fixations from interfering with the tendon markers. Thus any lengthening within the graft substance (due primarily to creep) between the fixations and the graft markers would lead to an overestimate in the lengthening in the regions of fixation. At the same time, the lengthening in the region between the fixations would be underestimated. To minimize the error introduced into lengthening in the regions of fixation by lengthening of the graft substance, the marker distance was 5 mm which was as small as possible practically. Because the maximum amount of lengthening in the region between the fixations over the approximately 70 mm distance between the graft markers was 0.23 mm, the lengthening per unit graft length was 0.003 mm/mm. Thus the lengthening in the regions of fixation were both overestimated by 0.015 mm or less than 2% relative error which is negligible. The lengthening in the region between the fixations was underestimated by 0.03 mm or approximately a 13% relative error. While the relative error in lengthening in the region between the fixations is larger, it can easily be reduced if not eliminated by correcting the reported value by 0.03 mm. Even with this correction, the lengthening in the region between the fixations was still substantially smaller than the lengthening in both regions of fixation.

Lengthening in the three regions was determined under constant amplitude cyclic loading, but in vivo the load transmitted to the graft would decrease as the graft construct lengthens. Although the ACL is the primary restraint to anterior knee laxity, previous studies have shown that knee stiffness increases with increasing anterior displacement in ACL deficient knees [13,14]. This increase in knee stiffness can only be due to increased support by the secondary structures. An increase in length of the graft construct would cause the anterior tibial displacement to increase thus bringing these secondary structures more into play. As more support is provided by the secondary structures, the tension in the ACL graft would be expected to decrease. Therefore, the methods used in this study overestimated the amount of lengthening particularly in the regions of fixation that would occur in vivo.

Lengthening in the region between the fixations determined in our in vitro study may not be representative of lengthening in this region that occurs in vivo. Animal studies have shown that graft remodeling, which occurs during postoperative healing, reduces the mechanical properties of the graft [24]. Animal studies have also shown that grafts become more susceptible to creep [2]. The reduction in mechanical properties in conjunction with an increased susceptibility to creep may result in increased stretching of the graft during loading, thereby lengthening the substance of the graft.

The number of loading cycles used in this study was based on the length of time that the graft might be cyclically loaded while the biological bond develops between the graft and bone tunnel. Animal studies have shown that a biologic bond develops between 4 and 8 weeks after surgery with the result that the transmission of load to the fixation device is reduced 85% [17,27]. Additionally, a patient recovering from reconstruction of the ACL typically experiences pain and swelling that limit mobility during the first 2 weeks. Thus lengthening of the graft construct due to lengthening in the regions of fixation can be expected to occur for 6 weeks postoperatively as a consequence of cyclic loading. Six weeks of normal activity corresponds to approximately 220,000 loading cycles for the ACL [20]. Therefore, the ACL grafts were loaded for 225,000 cycles to mimic 6 weeks of normal activity during rehabilitation.

To complete this number of loading cycles in a reasonable amount of time (i.e. 1 day), the frequency was set at 8 Hz, which is greater than the approximate 1.5 Hz/stride frequency of walking. Because creep is the primary mechanism of lengthening between the fixations and because creep is time dependent, the time course of the creep response in our in vitro tests may not be representative of the time course of the creep that would occur in vivo.

The rigid cross-pin used to model the femoral fixation was based on typical devices and methods used to reconstruct the ACL with a double-looped tendon graft. The diameter and width of the cross-pin were the same as that of the bone mulch screw (Arthrotek, Warsaw, IN) and the width of a typical femoral tunnel respectively. The actual size of the femoral fixation devices and femoral tunnels used in vivo may vary. Because one mechanism of lengthening in the region of the femoral fixation is a result of localized stress on the tendon caused by its contact as it loops around the cross-pin in the femoral tunnel (i.e. contact deformation), the amount of contact deformation may depend on the diameter of the cross-pin and size of the femoral tunnel.

Importance and interpretation of results

The RSA technique was a useful method for measuring the lengthening in all three regions of the graft construct in this in vitro study. Although this study was designed to determine the lengthening in the region between the fixations 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 in the regions between the fixations could occur beyond the time required for biological incorporation of the graft in the bone tunnels, determining lengthening in the region between the fixations 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 graft substance in the intraarticular space. However, the RSA technique used in this study for measuring lengthening in the regions of fixation could be applied in vivo.

The regions of fixation are potentially important to lengthening of double-looped tendon graft constructs used to reconstruct the ACL. Lengthening in each region alone may not result in a clinically important increase in anterior knee laxity, but the combined effect resulted in an increase in graft construct length of approximately 1.7 mm. Because the increase in graft construct length results in a roughly equal increase in anterior knee laxity [4], the increase in graft construct length from the combined contributions of both regions would manifest as a 1.7 mm increase in anterior knee laxity provided that the increase in graft construct length can not be recovered. A 1.7 mm increase in anterior knee laxity is more than half of the 3 mm increase that is used by clinical studies to characterize a recurrence of knee instability [10,11,16,25].

In considering whether the increase in graft length construct can be recovered, possible mechanisms of lengthening in each of the regions of fixation should be considered in turn. Because lengthening in the region of the tibial fixation most likely occurs as a consequence of failure at the graft–fixation device interface [12], any lengthening in this region can not be recovered. As noted earlier, the femoral fixation consisted of a cross-pin fixed to a rigid support. The primary mechanism responsible for lengthening in this region is contact deformation, which is a viscoelastic response of the tendon to the localized stress at the femoral fixation. Thus the corresponding lengthening will not recover while the graft is in tension. Because most double-looped grafts are fixed intraoperatively while under tension [6,26], because graft tension increases as the knee is flexed and extended during passive motion [23], and because rehabilitation activities create tension in the graft [21], it is unlikely that lengthening due to contact deformation can be recovered. Accordingly lengthening in both regions of fixation will translate into permanent lengthening of the graft construct and therefore will manifest as a permanent increase in anterior knee laxity.

The majority of lengthening in the regions of fixation occurred relatively early in the cyclic loading protocol (Fig. 4). This behavior indicates that some amount of lengthening of the graft construct will occur in aggressive rehabilitation programs as a consequence of early motion and weight bearing. Because biological incorporation of the graft in the bone tunnel requires up to 8 weeks as noted above [17], it seems unlikely that lengthening of the graft construct can be prevented completely in aggressive rehabilitation at least for the fixation methods tested herein. However the lengthening for the fixation devices tested herein is substantially less for a greater number of loading cycles than the lengthening for different fixation devices tested previously under constant amplitude cyclic loading [7]. Thus the choice of fixation methods should be carefully considered when reconstructed knees are to be aggressively rehabilitated and fixation methods which minimize lengthening of the graft construct under cyclic loading should be selected for these knees.

Lengthening in the region of fixation depends not only on the fixation devices used, but also on the graft type. A comparison of two graft types found a significant difference in lengthening in the region of fixation [9]. Because different combinations of fixation devices and graft types are used operatively, the contributions of lengthening in the region of fixation to lengthening of the graft construct should be determined separately for each combination. This can be accomplished by using the RSA technique demonstrated in this study with different graft constructs.

Lengthening in the region between the fixations contributed least to the increase in length of the grafts in this in vitro study. Lengthening in the region between the fixations was significantly less than that in the other two regions for all but the first 1000 cycles. Moreover, the pattern of lengthening was inconsistent in that lengthening did not monotonically increase with the number of loading cycles applied. The lack of a monotonic increase may have been because the random error of 0.2 mm in the measurement of all quantities including creep exceeded the average value of this quantity until after 100,000 cycles. In any case, the average lengthening in the region between the fixations after 225,000 cycles was less than 0.25 mm which was small in relation to the lengthening in both regions of fixation. As mentioned earlier however, lengthening in the in vitro environment may not be representative of the lengthening that occurs in the in vivo environment. Consequently lengthening in the region between the fixations may contribute to an increase in anterior knee laxity that occurs postoperatively.

The relative frequency with which the graft constructs in this study increased in length to clinically important levels is consistent with the relative frequency of clinically important increases in anterior knee laxity following ACL reconstruction. Although the total lengthening of the graft construct from the two regions of fixation reached 1.7 mm on average, the lengthening in both regions stabilized and the corresponding laxity increase (1:1) would be less than the 3-mm threshold used to identify a clinically unstable knee. However, in one of the ten specimens tested, the change in length of the graft construct was greater than 3 mm. This matches the 10% to 20% relative frequency of recurrent knee instability found in patients [10,11].

In summary, this study demonstrated the feasibility of using RSA to determine lengthening in each of three regions of double-looped hamstrings graft constructs cyclically loaded to 225,000 cycles. As far as we know, this study is the first to quantify lengthening of the graft construct in the region of a cross-pin fixation. Lengthening in the regions of fixation for the devices used in this study contributed the most to the lengthening of the graft construct, with maximum average values of 0.91 and 0.76 mm respectively after 225,000 cycles. Lengthening in the region between the fixations contributed least to lengthening of the graft construct in this in vitro study, with a maximum average value of 0.23 mm. Lengthening in both regions of fixation stabilized well before biological incorporation of the graft occurs and their combined effect is large enough to cause a recurrence of instability in some knees reconstructed with double-looped tendon grafts.

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