How Cyclic Loading Affects the Migration of Radio-Opaque Markers Attached to Tendon Grafts Using a New Method: A Study Using Roentgen Stereophotogrammetric Analysis (RSA)

An increase in anterior laxity following reconstruction of the anterior cruciate ligament (ACL) can result from lengthening of the graft construct in either the regions of fixation and/or the region of the graft substance between the fixations. RSA could be a useful technique to determine lengthening in these regions if a method can be devised for attaching radio-opaque markers to soft tissue grafts so that marker migration from repeated loading of the graft is limited. Therefore, the objectives of this study were 1) to develop a method for attaching radio-opaque markers to an ACL graft that limits marker migration within the graft, 2) to characterize the error of an RSA system used to study migration, and 3) to determine the maximum amount of migration and the time when it occurs during cyclic loading of ACL grafts. Tendon markers were constructed from a 0.8-mm tantalum ball and a stainless steel suture. Ten double-looped tendon grafts were passed through tibial tunnels drilled in bovine tibia and fixed with a tibial fixation device. Two tendon markers were sewn to one tendon bundle of each graft and the grafts were cyclically loaded for 225,000 cycles from 20 N to 170 N. At specified intervals, simultaneous radiographs were obtained of the tendon markers and a radiographic standard of known length. The bias and imprecision in measuring the length of the radiographic standard were 0.0 and 0.046 mm respectively. Marker migration was computed as the change in distance between the two tendon markers along the axis of the tibial tunnel. Marker migration was greatest after 225,000 cycles with a root mean square (RMS) value of less than 0.2 mm. Because the RMS value indicates the error introduced into measurements of lengthening and because this error is small, the method described for attaching markers to an ACL graft has the potential to be useful for determining lengthening of ACL graft constructs in in vivo studies in humans. [DOI: 10.1115/1.1644568]
rors are due to several sources that include system calibration, image quality, and the measurement of image coordinates, and are system dependent [12]. To determine whether marker migration occurs, any change in the coordinates due to migration must be distinguished from a change due to inherent measurement error. Therefore, the second objective of this study was to characterize the error of an RSA system used to study marker migration.

Because the error in measuring lengthening of graft constructs in vivo depends on the amount of marker migration that occurs postoperatively, the amount of migration must be determined for a particular method of attaching the markers to ACL grafts. Because migration could occur from cyclic loading of the grafts, a third objective of the study was to determine the maximum amount of migration and the time when it occurs during cyclic loading of soft tissue ACL grafts.

Materials and Methods

Construction of Tendon Markers. Markers were attached to soft tissue ACL grafts via stainless steel sutures. To construct each marker-suture complex (i.e., tendon marker) a tantalum marker was encased within a wire cage formed from a stainless steel suture. To secure the tendon markers to the soft tissue ACL graft, each tendon marker was sewn to the surface of the tendon bundle via the stainless steel suture. The details of this procedure are described below.

A set of four marker holders was used to construct the tendon markers. Each marker holder was made from 6.35-mm square aluminum stock cut to a length of 20 mm (Fig. 1). The marker holders were labeled from 1 to 4. A hole was drilled 0.3 mm deep and 0.8 mm in diameter and centered 0.5 mm from the end of marker holders 1 and 2. A 0.5 mm wide slot, 0.5 mm deep and 5 mm long, was machined at the end of one side of marker holders 3 and 4.

Each tendon marker was constructed from a tantalum marker and stainless steel suture using the set of marker holders (Fig. 2). A tantalum marker (0.8 mm diameter tantalum ball, Biomet Orthopedics Inc., Warsaw, IN) was placed in the spherical indentation of marker holder 1. Then marker holder 2 was aligned with the spherical indentation on top of the tantalum marker so that the marker was held between the marker holders. With the marker holders held together by a c-clamp, a stainless steel suture (size 4-0 with needle on each end, Ethicon, Somerville, NJ) was passed between the two marker holders and pulled around the tantalum marker. The free ends of the suture were wrapped 360 degrees about each other. Then marker holder 2 was aligned on top of marker holder 1. (c) A stainless steel suture was passed between the two marker holders and pulled around the tantalum marker. (d) The free ends of the suture were wrapped 90 degrees about each other. (e) The suture surrounding the tantalum marker was placed in the slot of marker holder 3. (f) Marker holder 4 was then aligned on top and the free ends of the suture were pulled around the tantalum marker a second time. (g) The free ends of the suture were wrapped 360 degrees about each other.

Specimen Preparation. To provide a tibial tunnel that simulated the tunnel created in an ACL reconstruction, 10 tibias were harvested from fresh-frozen bovine knees. Bovine tibias were used due to availability, low cost, and because they have been used by previous studies as a model of young human tibias [6,13]. 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 suture and marker were immersed in alcohol and wiped clean to remove the adhesive. The surrounding suture was then spot-welded to the tantalum marker at two places to complete construction of the tendon marker (Fig. 3).
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. 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 of the six markers (T1 and T2, Fig. 4) were placed in the tibia on a line parallel to the axis of the tibial tunnel through a specially designed cylindrical guide tool. The diameter of the guide tool was 8.9 mm to fit snugly within the tunnel and the length of the guide tool was 50 mm. Two holes were drilled oblique to the axis of the guide tool so that the holes exited the surface of the guide tool 2 cm apart and along a line parallel to the axis of the tool. Two markers were placed through these holes in the posterior wall of the bone tunnel using the bead injector device. The markers were placed at an equal depth by regulating the depth to which the bead injector device was inserted into each hole. With the markers at equal depth, the line connecting the two markers was parallel to the axis of the tunnel.

The four remaining markers (T3–T6) were distributed in the proximal end of the tibia. Although only three markers are required to establish a coordinate system, the use of additional markers created an overdetermined system and reduced the error in determining the position of the segment [14].

Bovine tendons were used to construct 10 double-looped ACL grafts. Bovine tendons were used because the structural, 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 [9]. 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 sizing sleeve (Arthrotek, Warsaw, IN). Each end of both tendons was sewn with a #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 being fixed to the tibia, the ACL graft was positioned in the tibial tunnel and each bundle was equally tensioned. The graft was passed through the tunnel and looped around a post positioned proximal to the tibial plateau. The post 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. 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 marker migration, two tendon markers (G1 and G2, Fig. 4) were sewn to one bundle of each ACL graft. The tendon markers were separated by a distance of only 5 mm to minimize the amount of viscoelastic creep that occurred between them. Since the amount of creep in a 85-mm long double-looped bovine tendon graft is less than 0.2 mm during cyclic load tests (225 N peak load) and stabilizes over time [9], the amount of creep between the tendon markers was negligible. The tendon markers were placed on the surface of one bundle away from the wall of the tunnel to avoid friction with the bone tunnel (Fig. 5). To sew the tendon markers to the tendon bundle, the two needles of the suture were passed straight through the same cross-section, approximately 1.5 mm apart and perpendicular to the long axis of the bundle. The needles were pulled through the bundle until the tendon marker pressed against the surface of the bundle and the suture ends were tightly tied in a double knot at the opposite side of the bundle. Once the tendon markers were sewn to the tendon bundle, the weights were reapplied to tension the ACL graft. With the four graft bundles equally tensioned, the tibial fixation device was secured. A Washerloc consisting of a spiked washer and cancellous bone screw (Arthrotek, Warsaw, IN) was used to fix the ACL graft to the tibia. The Washerloc was used because the amount of graft lengthening in the region of the tibial fixation is less using this device than other tibial fixation devices [7].

A radiographic standard of measured length was constructed to determine the error of our RSA system. The standard consisted of two tantalum markers (S1 and S2, Fig. 4) separated by an aluminum shaft, forming a dumbbell. The diameter of each tantalum marker was measured ten times and the length of the standard was measured thirty times with a precision of 2.5 microns using a micrometer (Model 230, L. S. Starrett, Athol, MA). The average diameters and average length were computed and used to determine the distance between the centers of the tantalum markers, which was defined as the standard length. The 95% confidence interval of the mean value of the standard length was 3 microns, which is 16 times better than the 50-micron accuracy of the RSA method [10]. Therefore the standard length using the method de-
cyclically loaded from 20 N to 170 N at 8 Hz for 225,000 cycles. The maximum load was equal to the maximum force that has been estimated to occur in the ACL during level walking [15].

Simultaneous radiographs were taken at specified intervals during the test to study marker migration as a function of cycle number. Radiographs were taken after 100, 225, 500, 1,000, 2,250, 5,000, 10,000, 22,500, 50,000, 100,000, and 225,000 cycles. At each interval, the load on the ACL graft was returned to the initial load of 20 N and held for 20 seconds to allow the load to equilibrate. Then the displacement of the crosshead (and ACL graft) was held constant until the radiographs were taken.

After completing the cyclic loading test, the attachment of the tendon markers was inspected for integrity. The specimen was removed from the materials testing machine and the graft was detached from the tibia. Each tendon marker was visually inspected and prodded with a blunt tool to ensure that it had not become either detached or loosened.

**Data Analysis.** Analysis of the radiographs was performed using a customized system. A digital image was obtained of each radiograph (Fig. 4) using a back-lit scanner (Epson 1600, Epson America Inc., Long Beach, CA) operating 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-D coordinates of the centroid of each marker were measured from the digital image using a software program (Scion Image 1.0, Scion Corporation, Frederick, MD).

A custom program written in Matlab (version 5.3, The Mathworks Inc., Natick, MA) was used to compute the transformation of image coordinates to the laboratory coordinate system, the positions of the roentgen foci, and the 3-D position coordinates of all markers in both the specimen and ACL graft (i.e., object points). The procedures and equations used by the program have been published previously [10]. To compute the transformation of image coordinates to the laboratory coordinate system represented by the calibration cage, a minimum of four fiducial marks (tantalum markers in the calibration cage closest to the image plane) such that no three are co-linear was used to define the calibration parameters for each view. The distance between each transformed fiducial mark and its known position (i.e., radial error) was also computed as a check of the calibration. The positions of the roentgen foci were computed using the set of control points (tantalum markers in the calibration cage closest to the X-ray source). A set of projection lines was created between the transformed control points and their known positions. The Nelder-Mead simplex method was used to calculate the position of the roentgen focus by determining the point that minimizes the sum of squared distances from the point to each projection line in the set. To compute the 3-D position coordinates of the object points, one projection line for each of the two images was constructed for each object point. The Nelder-Mead simplex method was used to calculate the 3-D position coordinates of each object point by determining the coordinates that minimize the sum of squared distances from the object point to the two projection lines. Additionally, the distance between the two projection lines was calculated to ensure correct marker identification.

A subroutine of the Matlab program was used to transform the 3-D position coordinates of the object points to a local coordinate system in the tibia. From radiographs taken prior to cyclic loading, a tibial coordinate system was created using three tibia markers such that the x-axis was defined by the axis of the tunnel (and direction of load). The first tibia marker (T1, Fig. 4) was chosen as the tibial origin. The subroutine transformed the position coordinates of the object points 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 coordinates to the tibial coordinate system at each interval.
coordinate system to the tibial coordinate system for each subsequent set of radiographs. The Nelder-Mead simplex method was used again to compute the transformation from the laboratory 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. All further calculations were performed from position coordinates in the tibial coordinate system.

For each specimen and at each time interval, the error of our RSA system was determined using the 3-D position coordinates of the markers in the radiographic standard. From each set of radiographs, the distance $B$ between the markers in the radiographic standard was computed. The error in determining the distance $B$ was defined as the difference between the computed length using RSA and the standard length $SL$:

$$\text{error}_i = B_i - SL$$

where the index $i$ denotes the cycle interval. The bias and imprecision of our RSA system were calculated as the mean and standard deviation of the error respectively, using the data from all ten specimens.

Marker migration was defined as the change in distance between the tendon markers, computed along the axis of the tibial tunnel, that occurred after cyclic loading. Before cyclic loading and at each successive time interval, the vector $d$ joining the two tendon markers was determined (Fig. 7). The change in the vector $d$ for the $i$th cycle interval, denoted as $\Delta d_i$, was determined from

$$\Delta d_i = d_i - d_0$$

where $d_i$ was the vector determined from the $i$th cycle interval and $d_0$ was the vector determined before the cyclic loading was applied. The vector $T$, defining the axis of the tibial tunnel, was also determined at each cycle interval using the two markers placed in the posterior wall of the tibial tunnel. Marker migration at each cycle interval, denoted by $M_i$, was computed from

$$M_i = \langle \Delta d_i \rangle - T_i$$

where $\langle \Delta d_i \rangle$ was the mean vector of migration at the time interval when migration was the greatest and $T_i$ was the vector of the error inherent to the RSA.

Results

None of the twenty tendon markers attached to the 10 grafts were detached after 225,000 cycles. Only one of the twenty tendon markers was loosened slightly (i.e., $<1$ mm of play) but the tendon marker was not detached from the graft. The migration was greatest for this graft among the ten grafts tested.

The bias ($\pm 95\%$ confidence interval) in determining the distance between two markers using our RSA system was $0.002 \pm 0.008$ mm and was not significantly different from zero (p
Although migration of tendon markers within an individual graft did not exhibit a systematic pattern with increasing number of load cycles (Fig. 8), the amount of migration considering all grafts generally increased with increasing number of cycles so that the RMS value was greatest at 0.192 mm after 225,000 cycles (Fig. 9). The mean value of migration after 225,000 cycles was 0.040 mm and was not significantly different from zero ($p = 0.538$). The F-test confirmed that the variance of the migration after 225,000 cycles was significantly greater than the error of the RSA system ($p<0.001$).

Discussion

To develop and validate an experimental method, which can be used to determine the causes of increased knee laxity in patients who have had an ACL reconstruction, the objectives of this study were 1) to develop a method for attaching tantalum markers to soft tissue grafts, 2) to characterize the error of an RSA system that was to be used to study migration, and 3) to determine the maximum amount of marker migration and the time when it occurs during cyclic loading of soft tissue grafts. The key results were that 1) a method for attaching tantalum markers was developed which maintained the attachment for 225,000 cycles of cyclic loading, 2) that the error of the RSA system was limited to 0.046 mm, and 3) that the maximum amount of migration measured for 10 soft tissue grafts was less than 0.2 mm and occurred at 225,000 cycles. Each of these key results is discussed in turn below.

In developing a method for attaching tantalum markers to tendon grafts, the materials used to construct the tendon markers and the method of their attachment to the graft were chosen specifically to determine lengthening in the regions of fixation and lengthening in the region between the fixations in vivo. Previous studies have implanted tantalum markers in tendons, but either the results of implanting the markers were not given or the applicability of the method for an in vivo study was not established [16,17]. These previous studies embedded the markers within longitudinal incisions in the tendons. In doing so, they may have created a channel through which the markers could migrate however. We chose to attach the tendon markers to the tendon rather than within the tendon to eliminate the potential for marker migration within the incision.

Inspection of the grafts after completion of the test showed that all tendon markers remained attached to the graft. This result testifies to the durability of the new method for attaching markers to soft tissue ACL grafts. Because this method can be used to determine graft lengthening in the regions of fixation and/or lengthening in the region between the fixations in vivo without loss of tendon markers and with limited amounts of migration, redundant markers are not warranted.

Galvanic corrosion resulting from combining the dissimilar metals of tantalum and 316L stainless steel will not be problematic in the in vivo environment for three reasons. One reason is that the difference in corrosion potentials for these two materials is small [18], thereby reducing the driving force for galvanic corrosion. A second reason is that reactive metals such as tantalum are generally not susceptible to galvanic corrosion because of their passive films [19]. A final reason is that even if some galvanic corrosion did occur, then the tantalum would be more affected than the stainless steel because it is more active in the galvanic series [18]. Consequently the stainless steel cage will experience reduced corrosion so that the mechanical integrity of the tendon marker should not be affected.

The placement site of the tendon markers on the ACL graft is an important factor that affects marker migration. A pilot study showed that placement of the tendon markers outside the designated placement region (Fig. 5) on the outer part of the double-looped graft resulted in higher levels of migration, presumably due to friction between the marker and the wall of the tibial tunnel. Thus to achieve the low levels of migration reported in this study, the tendon markers must be placed in the central part of the double-looped graft.

The method developed in this study for attaching tendon markers could also be used with other types of soft tissue grafts. Lengthening in the regions of fixation and/or in the region between the fixations could be determined from a single-looped graft using the same technique for construction of the tendon markers and attachment to the graft. However, the tendon markers should be placed in the central part of the graft, away from the wall of the tibial tunnel, to achieve the small amounts of migration reported in this study.

Our RSA system performed without bias and with an imprecision of only 0.046 mm. The error in RSA is system dependent because of such factors as variable image quality. The use of bovine tibias in which the bone is denser than that of human tibias [20] resulted in relatively poor image quality in our study. With the denser bone, it was more difficult to accurately identify the image coordinates in the radiographs. Because error in the measurement of image coordinates has the greatest influence on the error in determining a marker’s 3-D position coordinates [21], the imprecision value achieved in this study is a worst case for error in studies in humans.

The performance of our RSA system, which includes custom software used to compute the 3-D coordinates of markers, compares well with other RSA systems that have been used by previous studies. The traditional RSA system, which uses a 2-D mechanical measurement table and camera to measure image coordinates, determines 3-D positions of markers with an imprecision ranging from 0.030 mm to 0.050 mm under ideal conditions [10]. Because the distance between two markers on their 3-D positions, the error in determining the distance between two markers can be estimated from

$$
\sigma_{\text{dist}} = \sqrt{\sigma_{\text{pos}}^2 + \sigma_{\text{pos}}^2}
$$

where $\sigma_{\text{dist}}$ is the imprecision in determining the distance between two markers and $\sigma_{\text{pos}}$ is the imprecision in determining the 3-D positions of a marker. Using the results of Selvik [10] in the above equation, the imprecision in determining the distance between two markers using a traditional RSA system ranges from 0.042 mm to 0.070 mm and therefore is comparable to our value of 0.046 mm.

Recent advances in computer speed and storage capacity have led to the development of new digital RSA systems. These systems are similar to ours in that they use digitized radiographs from a scanner but differ in the computational software. A recent study introduced a digital RSA system that automatically detects markers in the digitized radiographs [22]. In this study, an imprecision of 0.030 mm was found in determining the distance between two markers. Though less than our imprecision of 0.046 mm, the imprecision by Vrooman et al. was obtained with ideal image quality from a radiographic phantom constructed of plexiglass, whereas our value was obtained under non-optimal conditions that would be more representative of an in vivo study. Additionally, our calibration errors were typically much less than the mean calibration errors found by their study. Therefore, the performance of our RSA system compares well with that of recent digital RSA systems.

Because the F-test showed that migration could not be attributed to the error of the RSA system alone, marker migration did occur. Accordingly, migration must be viewed as a source of error in determining lengthening in the regions of fixation or lengthening in the region between the fixations. However, this study showed that the migration increased gradually as the number of loading cycles increased so that the maximum error occurred at 225,000 cycles. Moreover migration was unbiased with an imprecision of less than 0.2 mm. The imprecision represents a source of random error that would be introduced into measurements of
lengthening in the regions of fixation and/or in the region between the fixations. Thus the error in these quantities introduced by migration is bounded by the value at 225,000 cycles. Accordingly, the method of attachment of markers for soft tissue grafts is suitable for longitudinal studies and smaller errors than 0.2 mm can be expected at time points where the number of loading cycles is less than 225,000.

Marker migration was defined as the change in distance between the two markers projected along the axis of the tibial tunnel rather than the change in distance between the two markers. With the definition selected, migration would manifest as an error in measuring either lengthening in the regions of fixation or lengthening in the region between the fixations. With the alternative definition, any migration of the markers within the cross-section of the tendon would affect the computed value of migration but would not represent an error in determining either lengthening in the regions of fixation or lengthening in the region between the fixations.

To keep the number of markers in the graft to a minimum, only two tendon markers were attached to a tendon bundle. With this arrangement, the marker migration either could have been underestimated if both tendon markers migrated in the same direction or could have been overestimated if both tendon markers migrated in opposite directions. Because overestimations would inflate the amount of migration as defined by the RMS value, the imprecision determined in this study represents a worst-case value.

The number of loading cycles used in this study was based on the length of time that the graft might be cyclically loaded during rehabilitation following ACL reconstructive surgery. Animal studies have shown that a biologic bond between the graft and bone tunnel develops between 4 and 8 weeks after surgery with the result that the transmission of load to the fixation device is reduced 85% [23,24]. Additionally, a patient recovering from reconstruction of the ACL typically experiences pain and swelling that limit mobility during the first 2 weeks. Thus marker migration 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 [25]. Therefore, the ACL grafts were loaded for 225,000 cycles to mimic 6 weeks of normal activity during rehabilitation. The loading frequency was chosen to complete the test during 1 day.

This study did not investigate causes other than cyclic loading that could lead to marker migration. Graft remodeling, which occurs in the biological environment and affects the mechanical properties of the graft [26], may also affect the integrity of the attachment of tendon markers. However, remodeling occurs from 2 to 10 months after reconstruction [27], which is after the expected time required for biologic incorporation of the graft in the tibial tunnel [23]. Because lengthening in the regions of fixation need only be measured until biologic incorporation occurs, marker migration due to graft remodeling would not affect this measurement. Thus, when viewed on the whole, the preceding discussion establishes the potential of the method of attaching tendon markers to soft tissue grafts described herein for measuring lengthening in the regions of fixation in the in vivo environment.

Using the method of attaching tendon markers to soft tissue grafts for direct measurement of lengthening in the region between the fixations has less potential for application in the in vivo environment, however. Tendon markers attached in the intraarticular portion of the graft may be affected by marker migration due to graft remodeling. Additionally, a dislodging of the tendon marker in the intraarticular space could cause damage to the articular surfaces. Finally the graft is not straight in general so that determining lengthening in the region between the fixations becomes more complicated as a direct measurement.

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References

