Migration of Radio-Opaque Markers Injected Into Tendon Grafts: A Study Using Roentgen Stereophotogrammetric Analysis (RSA)

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An increase in anterior laxity following reconstruction of the anterior cruciate ligament (ACL) can result from lengthening of the graft construct either at the sites of fixation and/or between the sites of fixation (i.e., graft substance). Roentgen stereophotogrammetric analysis (RSA), which requires that radio-opaque markers be attached to the graft, has been shown to be a useful technique in determining lengthening in these regions. Previous methods have been used for attaching radio-opaque markers to the graft, but they all have limitations particularly for single-loop grafts. Therefore, the objective of this study was to evaluate injecting markers directly into the substance of a tendon as a viable method for measuring lengthening of single-loop graft constructs by determining the maximum amount of migration after cyclic loading. Tantalum spheres of 0.8 mm diameter were used as tendon markers. Ten single-loop tendon grafts were passed through tibial tunnels drilled in calf tibias and fixed with a tibial fixation device. Two tendon markers were inserted in 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. Marker migration was computed as the change in distance between the two tendon markers parallel to the axis of the tibial tunnel. Marker migration had a root mean square (RMS) value of less than 0.1 mm. Because the RMS value indicates the error introduced into measurements of lengthening and because this error is negligible, the method described for attaching markers to single-loop ACL grafts has the potential to be useful for determining lengthening of single-loop ACL graft constructs in in vivo studies in humans.
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Introduction

Knee instability following anterior cruciate ligament (ACL) reconstruction manifests as an increase in anterior laxity and occurs in 10%–20% of cases [1–5]. Although the cause(s) is unknown, increased anterior laxity can be traced to lengthening of the graft construct (i.e., graft-fixation-bone complex). Two possible causes of lengthening of the graft construct are lengthening at the sites of fixation [6–8] and lengthening between the sites of fixation (i.e., graft substance) [8,9]. Knowledge of the amount and timing of lengthening at the sites of fixation and between the sites of fixation 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 graft construct lengthening that occurs postoperatively.

One method that offers potential for study of graft construct lengthening is Roentgen stereophotogrammetric analysis (RSA). This method has been explained in detail previously [10–12]. The attraction of using RSA to study graft construct lengthening is the 50 μm error with which distances between two markers can be determined [13]. To achieve this error however, any markers attached to the graft must not migrate as the graft construct is cyclically loaded. A previous study devised a method that attached markers to soft tissue grafts by means of stainless steel sutures and demonstrated that migration was bounded by a RMS value of 0.2 mm [13]. To achieve this RMS value of migration however, markers must be placed in the central part of graft so that they are not exposed to the tunnel wall [13]. While this requirement can be satisfied for a four-bundle or double-loop tendon graft, it is more difficult to satisfy for a two-bundle or single-loop graft. Because single-loop grafts such as those constructed from tibialis tendons are gaining popularity based on favorable clinical reports [14,15], it would be advantageous to develop an attachment method for this graft type. Therefore, the objective of this study was to evaluate direct injection of markers in single-loop grafts as a viable method for measuring lengthening of graft constructs by determining the maximum amount of migration after cyclic loading. If the migration was small relative to the amount of lengthening of clinical importance (>1 mm), then direct injection could be considered a viable method.

Materials and Methods

Specimen Preparation. To provide a tibial tunnel that simulated the tunnel created in an ACL reconstruction, ten tibias were harvested from fresh-frozen calf knees. Calf tibias were used due to availability, low cost, and because they have been used by previous studies as a model of young human tibia [6,13,16–18]. 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 (PMMA) so that the proximal end remained accessible for drilling a tibial tunnel.

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 65 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. The markers (0.8 mm diameter tantalum balls, Tilly Medical Products AB, Lund, Sweden) were implanted in the tibia using a marker injector device (Tilly Medical Products AB, Lund, Sweden). Two (T1 and T2, Fig. 1) of the six markers were placed in the tibia on a line parallel to the axis of the tibial tunnel through a specially designed cylindrical guide tool [13]. The four remaining markers were distributed in the proximal end of the tibia. The use of six markers created an overdetermined system and reduced the error in determining the position of the

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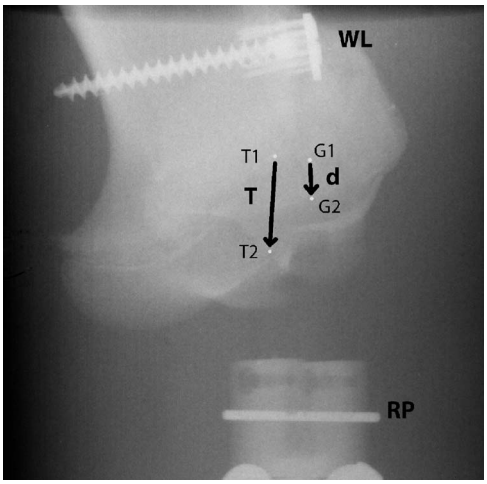

Fig. 1 Scanned radiograph of proximal calf tibia and ACL graft showing the WasherLoc tibial fixation device (WL), tendon markers (G1 and G2), markers placed along the axis of the tibial tunnel (T1 and T2), and the rigid post (RP). The remaining four markers inserted into the tibia and used to define the local tibial coordinate system have been omitted for clarity. The vectors d and T are indicated.

markers [10].

Bovine tendons were used to construct ten single-loop ACL grafts because properties of single-loop grafts constructed from these tendons are similar to those of grafts constructed from human tendons [9,19]. For each graft, the middle extensor tendon was harvested from a bovine forelimb of a skeletally mature animal. The tendon was cut at the bifurcation and trimmed so that the folded single-loop graft just passed through a 9-mm 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.

An injector device was used to place two tendon markers within the substance of the tendon graft (G1 and G2, Fig. 1). The injector device was oriented perpendicular to the long axis of the tendon. The needle was then inserted approximately $\frac{3}{4}$ across the diameter of the tendon. One marker was injected into the tendon and the injector device was removed. The second marker was injected 5 mm along the length of the tendon from the first marker. Keeping the intermarker distance at 5 mm resulted in a negligible viscoelastic creep of 0.016 mm, which was calculated based on the 0.3 mm of creep in a 95-mm long single-loop anterior tibialis tendon graft during constant load tests at 250 N [19].

The graft was positioned in the tibial tunnel, tensioned, and fixed. The graft was passed through the tunnel and looped around a post which was positioned in line with the tunnel at a distance of 25–30 mm from the tibial plateau due to the differences in tunnel length. The length of the graft from the proximal end of the loop to the distal end of the tibial tunnel was standardized to 75 mm. The two limbs of the graft were equally tensioned from the distal opening of the tunnel using a custom jig and hanging 10 N weights on each limb [13]. An extended spike WasherLoc consisting of a spiked washer and cancellous bone screw (Arthrotek, Warsaw, IN) was used to fix the graft to the tibia. This device was used because the amount of lengthening of the site of the tibial fixation is less than other tibial fixation devices [7].

Experiments. Cyclic loading of the ACL grafts was applied by a materials testing machine (Table Top 858, MTS Corporation, Minneapolis, MN) using methods described previously [13]. Briefly, each tibia was placed in the machine such that the axis of the tibial tunnel was the same as the axis of loading. The graft was looped around a 2.5-mm diameter post fixed to the base of the machine and was immersed in a saline bath (0.9% isotonic solution) at room temperature. Made from plexiglass and modified to hold two x-ray cassettes, the calibration cage (Model 10, Tilly Medical Products AB, Lund, Sweden) surrounded the tibia and ACL graft so that all calibration markers embedded in the plexiglass could be seen from each view. While the materials testing system applied a tension of 20 N to the ACL grafts, simultaneous radiographs were obtained to record the initial positions of the tendon markers. Then the ACL grafts were cyclically loaded from 20 N to 170 N at 10 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 [20]. Simultaneous radiographs were taken after 100, 225, 500, 1000, 2250, 5000, 10000, 22500, 50000, 100000, and 225000 cycles. At each interval, the load on the ACL graft was returned to the initial load of 20 N and held for 20 s to allow the load to equilibrate. Then the displacement of the crosshead (and ACL graft) was held constant while the radiographs were taken. The total time to complete the testing on one graft was approximately 10 h.

Data Analysis. Analysis of the radiographs was performed using a customized system described previously [13]. Using this system, the three-dimensional coordinates of object points were determined. Marker migration was defined as the change in distance between the tendon markers, computed parallel to the axis of the tibial tunnel, that occurred after cyclic loading. Marker migration at each cycle interval, denoted by M_i was computed from

$$M_i = \frac{\Delta \mathbf{d}_i \cdot \mathbf{T}_i}{|\mathbf{T}_i|} \tag{1}$$

where $\Delta \mathbf{d}_i$ is the change in the vector $\mathbf{d}$ joining the two markers for the ith cycle interval from the vector before loading was applied and $\mathbf{T}_i$ is the vector defining the axis of the tibial tunnel. Positive migration was defined as an increase in the distance between the tendon markers.

Because the migration could be either positive or negative (i.e., increase or decrease in distance between tendon markers), the root mean square (RMS) value was computed to indicate the amount of migration at all cycle intervals and the maximum value was used to identify the interval at which the migration was the greatest. To determine whether migration was a random phenomenon, the mean value of the migration was determined at the time interval when migration was the greatest and was compared to zero using a t-test. To determine whether the variance of the migration was greater than the inherent error in determining the distance between two markers, an F-test was used to compare migration at the time interval when migration was the greatest with the variance of the error inherent to the RSA system which was $(0.046\ \text{mm})^2$ [13].

Results

Migration of tendon markers within an individual graft did not exhibit a systematic pattern with increasing number of load cycles (Fig. 2) and the RMS migration also did not exhibit a systematic pattern. The range of the migration over all load intervals was ±0.18 mm (Fig. 2) and the RMS migration was greatest after 1000 cycles of loading and reached a value of 0.085 mm (Fig. 3). The mean value of migration when migration was greatest was 0.006 mm and was not significantly different from zero ($p=0.826$). The F-test indicated that the variance of the migration when migration was greatest was marginally not significantly greater than the variance of the error inherent to the RSA system ($p=0.053$).

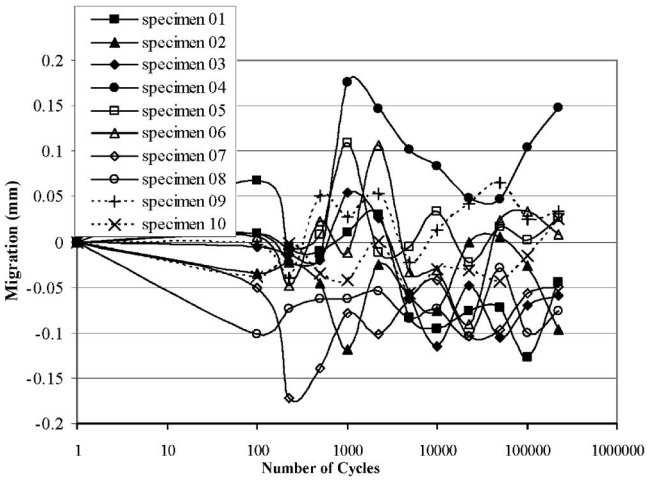

Fig. 2 Marker migration for all specimens plotted against the number of cycles (log scale)

Discussion

Because it is of interest to determine the causes responsible for lengthening of single-loop soft tissue graft constructs and because a previously described method is useful primarily for double-loop grafts constructs [13], the objective of this study was to evaluate direct injection of markers as a viable method for attaching markers in single-loop grafts. The key result was that the maximum RMS migration for ten grafts was less than 0.1 mm. Because the methods of testing were justified previously as well as causes of migration other than cyclic loading [13], the remainder of this section concentrates on the usefulness of our method and the importance of the key finding.

Although implanting markers directly into tendons has been described previously [21–23], our technique of direct injection differs in one potentially important aspect from previously described techniques. These previous studies implanted the markers within either longitudinal incisions or canals in the tendons. In doing so, they may have created a channel through which the markers could migrate along the length of the tendon. We chose to inject the markers perpendicular to the long axis of the tendon to minimize migration along the length of the tendon.

While translation along the length of the tendon (i.e., migration) was indeed minimized as indicated by the small RMS migration of 0.1 mm found in our study, the markers might have migrated along the channel created by the injector device, which was perpendicular to the long axis of the tendon. Migration of the markers along the transverse channel was evaluated by computing the translation along the channel and the maximum RMS value was limited to 0.15 mm indicating that translation along the channel was minimal.

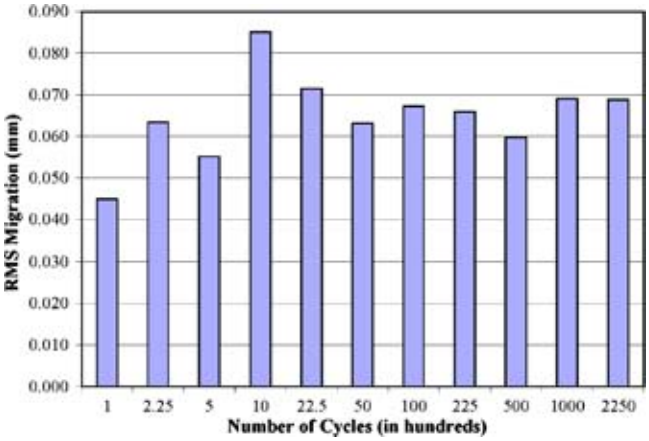

Fig. 3 Root mean square (RMS) value of migration plotted against the number of cycles

Our method of direct injection of markers into tendons also offers several advantages over alternative methods of attaching markers to tendons. One alternative method fabricated a special stainless steel suture which contained a marker [13]. Thus one important advantage of direct injection is simplicity; direct injection obviates the need for specialized tooling to fabricate a suture marker and for the time required in fabricating these markers. Furthermore direct injection also simplifies the attachment process in *in vivo* applications thus saving valuable time in the operating theater.

A second important advantage is versatility. Because the suture markers are external to the graft substance, care must be taken in attaching the marker to the graft so that the marker is not immediately adjacent to the tunnel wall. Roos et al. reported that if this placement was not achieved, then the migration increased substantially [13]. In a double-loop graft with four bundles, this placement can be achieved by insuring that the marker is opposed by the other bundles. However in single-loop grafts such as the tibialis tendon graft, achieving this placement is much more difficult because only a single bundle is available for opposition and even if this placement is achieved initially, then it may not be maintained as the graft is cyclically loaded. With direct injection however, the marker is necessarily imbedded into a bundle of the graft so that the marker is protected by the surrounding graft material without the need for careful placement.

A final advantage is that the RMS migration of direct injection was better than the RMS migration of the attachment of the marker with steel sutures. The RMS migration of the attachment of the marker with steel sutures was 0.2 mm, which is approximately twice that of markers directly injected perpendicular to the length of the tendon. The reduction in RMS migration with directly injected markers might be due to the elimination of the steel suture and any resulting friction between the steel suture-marker and tunnel wall that could cause displacements relative to the tendon with each load cycle.

Of the two possible causes of lengthening of the graft construct, (i.e., lengthening at the sites of fixation and lengthening between the sites of fixation), direct injection may not be suitable for determining lengthening between the sites of fixation. To determine lengthening between the sites of fixation, it would be necessary to implant markers in the portion of the graft spanning the intraarticular space for two reasons. One reason is that the graft is not straight in general so that the length of the graft cannot be determined solely from markers implanted near the fixation devices. A second reason is that remodeling of the graft occurs in 2–10 months [24] which is after the expected time required for biologic incorporation of the graft in the tibial tunnel [25,26]. Thus lengthening of the graft spanning the intraarticular space would need to be monitored after lengthening at the sites of fixation can no longer occur as a result of the biological incorporation of the graft in the bone. Because of the possibility that markers may migrate transversely to an extent where they dislodge from the tendon and become loose bodies in the joint space, direct injection of markers into the portion of the graft spanning intraarticular space is not recommended.

While this study focused on single-loop tendon grafts, it is of interest to consider whether direct injection of markers could also be used for double-loop grafts where each of the tendons has a smaller cross-sectional area [9]. To answer this question, markers were injected into the tendons used to form double-loop grafts. Due to the smaller cross-sectional area, about 25% of the markers injected did not remain in the tendon and an additional 25% could be seen visually in the channel created by the injector device.

Thus it is unlikely that direct injection of markers into tendons used to form double-loop grafts is a viable method.

In summary, our study has demonstrated that direct injection of markers into single-loop soft tissue grafts is a viable method for measuring lengthening of single-loop graft constructs. The migration associated with direct injection is both random in nature and sufficiently small (<0.1 mm) so that it adds minimal error to RSA measurements of length changes. Beyond the small error, other advantages of direct injection over alternative methods of attaching markers to tendons are simplicity and versatility. Considering these advantages, direct injection is a useful technique which is expected to find use in both cadaveric and in *in vivo* studies. In *in vivo* studies, directly injecting markers is useful for determining lengthening at the sites of fixation where the markers are contained within the bone tunnels but is not recommended for determining lengthening between the sites of fixation because of concerns that the markers may dislodge from the graft into the joint.

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