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How four and twelve weeks of implantation affect the strength and stiffness of a tendon graft securely fixed in a bone tunnel: a study of Evolgate fixation in an extra-articular model ovine model

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Abstract Healing of a tendon graft to a bone tunnel is slower than the healing of a bone plug. Therefore, the device chosen for hamstring fixation may need to maintain its strength and stiffness longer than the device chosen for bone-tendon-bone fixation. We evaluated, in an extra-articular ovine model, how 4 and 12 weeks of implantation affect the strength of a tendon graft fixed to bone with the Evolgate. The long digital extensor tendon was transplanted and fixed with the Evolgate into a 30-mm long, 8 mm diameter bone tunnel drilled in the tibial metaphysis of both posterior limbs of 15 skeletally mature Suffolk sheep. Immediately after implantation, and 4 and 12 weeks later, biomechanical cyclic load tests in 50 N increments were performed until failure to evaluate the ultimate failure load (UFL). Histological analysis was also performed at 4 and 12 weeks. Biomechanical tests revealed a UFL of 339±120 N at time 0, and increases to 635±19 N (4 weeks) and to 867±80 N (12 weeks). The differences between all 3 groups were significant ($p<0.001$, paired t test). The histological evaluation showed a layer of cellular, fibrous tissue between the tendon and the bone, along the length of the bone tunnel; this layer progressively matured and reorganized during the healing process. The collagen fibers that attached the tendon to the bone resembled Sharpey's fibers. The strength of the interface significantly and progressively increased between weeks 4 and 12 after transplantation, and was associated with a degree of bone ingrowth noted histologically. The use of the Evolgate seems not to interfere with the bone ingrowth after implantation, allowing an improvement in strength of the bone-tendon-device complex.

Key words ACL reconstruction • Biomechanics • Histology • Tendon • Tibial fixation

Introduction

Since the first description by Galeazzi [1, 2], reconstruction of the anterior cruciate ligament (ACL) with semitendinosus and gracilis tendons has become popular in the past decade, essentially because of the low harvest morbidity compared with patellar tendon. Moreover, a laboratory study [3] has shown that a combined four-strand hamstring graft is stronger and stiffer than a 10-mm patellar tendon graft. However, healing of a tendon graft to a bone tunnel is slower than healing of a bone plug, as that used with the patellar tendon graft [4]. Therefore, the device chosen for hamstring fixation may need to maintain its strength and stiffness longer than the device chosen for bone-tendon-bone fixation, especially on the tibial side, which is still considered the weak point of this technique. Although several studies [5] investigated the biomechanics of the initial strength and stiffness of various devices for tibial fixation of hamstring grafts, very few in vivo studies [4, 6–9] dealt with the effect of a fixation device on healing of a tendon graft to the bone tunnel and how strength and stiffness of the complex (tendon-device-bone) change after implantation.

In previous studies [10–12], we tested the Evolgate (Citieffe, Bologna, Italy), a fixation device designed to improve the biomechanical properties of the interference screw by reinforcing the walls of the tibial tunnel with an involute, a sort of spiral. The Evolgate is composed of three components made in a titanium alloy: an involute (21 mm in length, 10 mm in diameter) with a spike positioned at one extremity, a screw (20 mm long, 9 mm diameter) and a washer. The involute (coil) reinforces the walls of the distal half of the tibial tunnel, the screw interferes with tendons and the involute and the washer provide a cortical grip. The coil is inserted into the tibial tunnel using a special impactor (also acting as an extractor should a revision be necessary), which permits penetration of the spike in the pre-drilled tibial cortex. The tendons, secured at the femoral side, are pulled through the bone tunnel and the four ends of the tendons, coming out from the tibial side, are properly tensioned; the screw is then inserted, interfering with the tendons and the spiral, until the washer leans against the tibial cortex. The spike prevents rotation of the spiral as the screw tightens.

On the basis of several biomechanical tests conducted with both pull out and cyclic loading, the biomechanical properties of the Evolgate were initially found to be better than other aperture fixation devices such as the interference screw [10, 12] and Intrafix (DePuy-Mitek) [11]. The purpose of this study was to evaluate, in an extra-articular ovine model, how 4 and 12-weeks of implantation affect the strength of a tendon graft fixed to bone with the Evolgate. Since anchorage of hamstring tendons to bone is not yet clearly histologically understood, a second goal of this study was to histologically document, in the same ovine model, the type of healing of the tendon graft to the bone tunnel provided by Evolgate fixation, 4 and 12 weeks after implantation.

Materials and methods

Experiments were performed in 15 skeletally mature Suffolk sheep (30 limbs). The study was performed according to Italian law no. 116/92 concerning the use of animal specimens for experimental in vivo studies.

The long digital extensor tendon (about 7 mm in diameter) was transplanted into a 30-mm long, 8 mm diameter bone tunnel drilled in the tibial metaphysis of the posterior limbs and fixation was performed with the use of Evolgate (Citieffe, Bologna, Italy). Briefly, general endotracheal anaesthesia was achieved with inhalation agents. A 10-cm longitudinal incision was made lateral to the patellar tendon. The common digital extensor tendon was detached from the lateral femoral condyle (Fig. 1a). A 30-mm tunnel was placed obliquely across the dense metaphyseal bone of the proximal tibia. The coil of the Evolgate was inserted into the bone tunnel and the tendon was passed through the coil. The screw was completely turned (Fig. 1b) until the washer lay against the tibial cortex. The incision was closed in layers with absorbable sutures and the skin was closed with #2 absorbable sutures.

Surgical procedures on both posterior limbs were staggered in order to permit concurrent biomechanical and histological analyses 4 and 12 weeks after operation. Briefly, at the start of the study (time 0), 3 sheep were operated on both hind limbs and immediately sacrificed for biomechanical analysis. The remaining 12 sheep were operated on the right hind limb at time 0 and

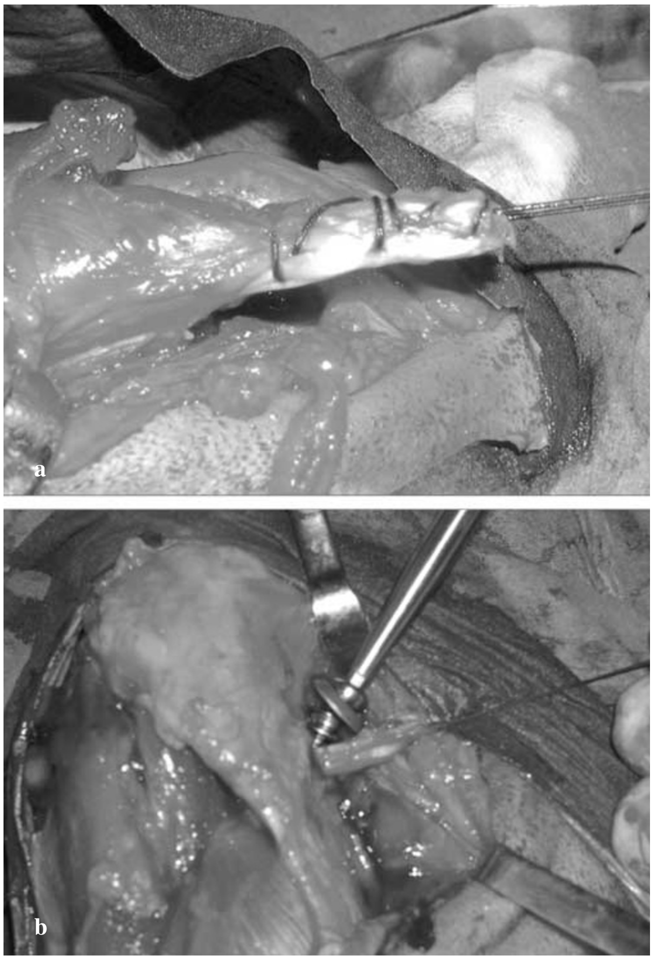

Fig. 1a, b *Surgical procedure*. **a** The common digital extensor tendon was detached from the lateral femoral condyle. **b** The screw was screwed inside the bone tunnel until the washer lay against the tibial cortex on the left hind limb 8 weeks later. These sheep were sacrificed 12 weeks after the start of the study, so that the left limb was analyzed 4 weeks after surgery (time 1) while the right limb was analyzed after 12 weeks (time 2). Of these 12 sheep, 6 were used for biomechanical studies, 3 for histological studies and 2 were excluded due to *Staphylococcus aureus* infection.

Sheep were killed by intravenous injection. The hind limbs were amputated above the knee and tagged for identification. The limbs were stored overnight at 0° C and thawed the next day for testing. In the group of specimens used for biomechanical evaluation, the Evolgate was not removed and tests were performed with the device inside the bone tunnel. In the group used for histological evaluation, the screw of the Evolgate was removed.

Biomechanical testing

Limbs were prepared for testing by disarticulating the tibia from the femur and removing all soft tissue from the tibia except for the common digital extensor tendon and muscle. Adhesions were sharply freed between the muscle-tendon unit and tibia to the tunnel entrance. Each tibia was mounted and fixed with wires in a 50 mm diameter cylinder. The complex was mounted on the tensile machine (model Z010, Zurich-Roell, Ulm, Germany), so that the bone tunnel axis was the same of the load applied to the tendon. The muscle, at the muscle-tendon junction, was fixed in a special cryo-jaw clamp that allows fixation and freezing of the muscle by dry-ice, to avoid muscle slipping during load application.

A 50 N preload for 10 seconds was applied. After preload, a cyclic load test in 50 N increments per cycle was performed until failure to evaluate the ultimate failure load. Data were recorded with dedicated software (Textexpert 8.1, Zwick-Roell) and were evaluated with a load-displacement curve. We evaluated the ultimate failure load (UFL) at time zero, time 1 (4 weeks) and time 2 (12 weeks).

Histological evaluation

A total of 6 tibias (three at time 1 and three at time 2) were used for histological study. The graft-tibia specimens were fixed in a 10% buffered formalin solution immediately after harvesting from each limb. After the specimen was decalcified, it was cast in paraffin blocks. The specimens were sectioned parallel and horizontal to the longitudinal axis of the bone tunnel, both in the section of the tunnel with the coil and in the section without the coil to evaluate the role of the coil in the healing process (Fig. 2). The specimens were stained with hematoxylin and eosin.

Statistical analysis

The unpaired *t* test was used to assess differences in UFL between limbs studied at time 1 and time 2. Moreover, the same test was used to compare UFL between time 0 animals and those studied at times 1 and 2. The difference was considered statistically significant if $p<0.05$.

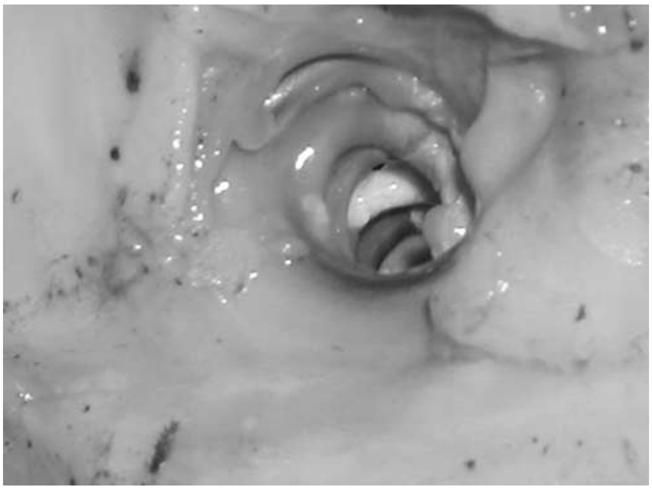

Fig. 2 The specimens were sectioned parallel and horizontal to the longitudinal axis of the bone tunnel, both in the portion of the tunnel with the coil and in the portion without the coil, to evaluate the role of the coil in the healing process

Fig. 3 Removal of the screw after four weeks of implantation (T1)

Results

After 4 and 12 weeks of implantation, the Evolgate screws were easily removed from those specimens destined for histological examination. After 4 weeks, the involute was covered by a fine reactive tissue (Fig. 3). After 12 weeks,

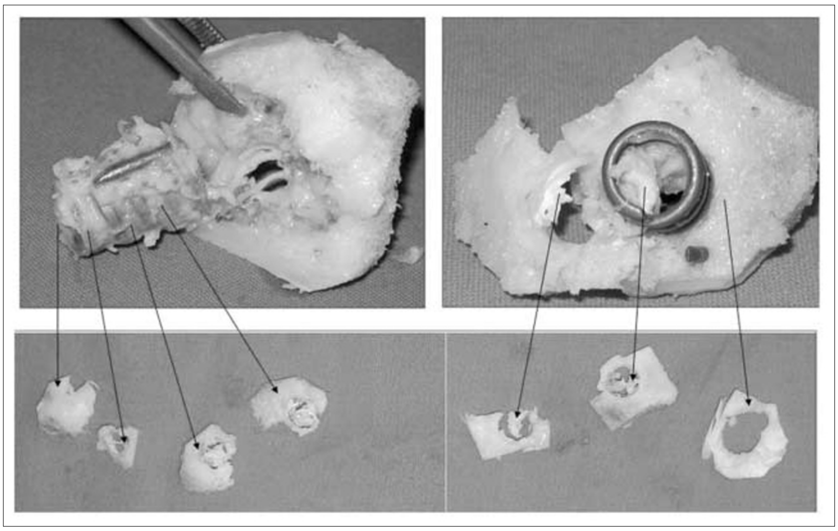

Fig. 4 Twelve weeks after implantation (T2), there was ingrowth of a white fibrous tissue between the spirals of the coil

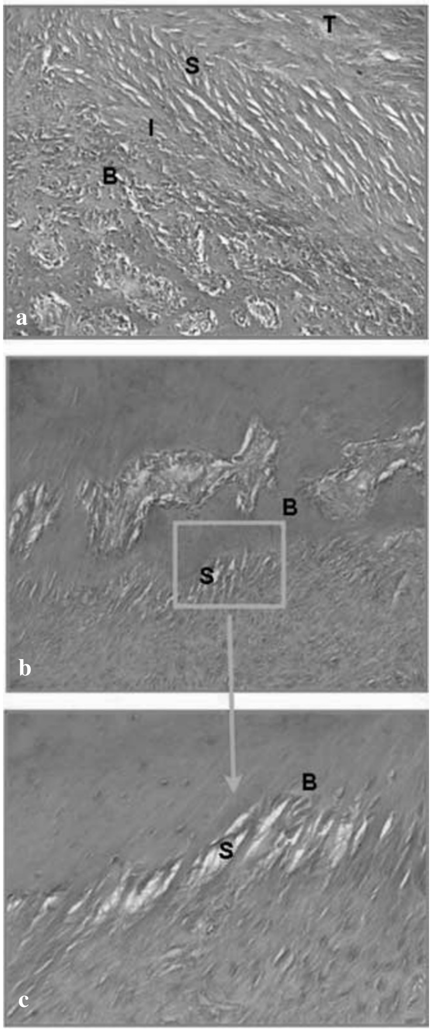

Fig. 5a-c Histological study of the tendon-bone interface after four weeks. **a** 100x magnification. **b** 200x magnification. **c** 400x magnification. *B*, bone; *T*, tendon; *I*, interface; *S*, Sharpey's fibers

Table 1 Ultimate failure load (pull-out strength) of a tendon graft fixed to bone using Evolgate, in sheep at 0, 4 and 12 weeks after operation. A total of 6 limbs were tested in each group

Group	Animals, n	Limbs	UFL, N[a]
Time 0	3	L + R	339 (134)
Time 1 (4 weeks)	6	L	635 (34)*
Time 2 (12 weeks)	6	R	867 (133)*†

*p<0.001 vs. time 0; † p<0.001 vs. time 1, paired *t* test
[a] Values are mean (SD)

we observed the ingrowth of a white fibrous tissue between the spirals of the coil (Fig. 5).

At time 0, the ultimate failure load (UFL) was 339±134 N (mean±SD); after 4 weeks of implantation it was 635±34 N while after 12 weeks of implantation it was 867±133 N. The differences in UFL were significant for all comparisons between groups (Table 1).

Regarding the histological evaluation of the tendon graft-bone tunnel interface (Fig. 5), 4 weeks after implantation, the granulation tissue had developed into a dense connective tissue with poor vascularization (Fig. 5a). A layer of cellular, fibrous tissue was noted between the tendon and the bone, along the length of the bone tunnel. Some activated osteoblasts were seen rimming the adjacent bone trabeculae. After 12 weeks (Fig. 5b, c), some perpendicular collagen fibers were observed at the interface between the tendon and the osseous tunnel. The collagen fibers that attached the tendon to the bone resembled Sharpey fibers.

Immediately after implantation (T0), all complexes failed by pullout of the tendon from the end of the tunnel. After 4 weeks of implantation (T1), 5 of 6 cases failed by pullout of the tendon from the end of the tunnel, and one case failed by rupture of the tendon outside the tunnel. After 12 weeks of implantation (T2), the tendon failed by rupture of the tendon outside the tunnel in all cases.

Discussion

One issue of the present paper is the use of a single fixation device instead of at least two devices, where one served as a control. We selected the Evolgate because it is one of the strongest and stiffest tibial fixation devices [11, 12].

A second issue is the length of the tunnel, which in the ovine model is shorter than in the human tibia. Using the standard 21-mm Evolgate, in the present study we left a smaller portion of tendon "free inside the tibial tunnel", namely uncompressed against the bony walls of the tunnel (a few millimeters instead of about 1.5 cm as occurs in human fixation). However, because the strength of the interface between a tendon graft and bone tunnel increases with the length and the surface of the tunnel [13], a larger area of bone-tendon contact could eventually improve the tendon to bone healing, resulting in an even more favorable condition in humans.

A third, and probably a major issue of this study is the use of an extra-articular model. Although this model has some advantages such as avoiding variables associated with intra-articular ACL graft positioning and tensioning and allowing the testing on only one site of fixation, the healing environment in the extra-articular model may be different from that of the intra-articular model; in fact, as previous authors speculated, the penetration of synovial fluid into the tibial tunnel could affect and alter the healing process, by diluting the initial hematoma and preventing fibrin clot formation [14].

If a secure, initial graft fixation is important for the success of ACL reconstruction, the development of a strong and stiff biological attachment of the tendon graft to the bone tunnel in the postoperative period is even more important, for rehabilitation and resumption of athletic activities. The factors that influence the healing of a tendon within a bone tunnel are many and include the amount of the tendon within the tunnel at the time of surgery, the initial fit of the tendon within the tunnel, the length of time from implantation and the fixation methods used. To our knowledge, only one study [9] has compared the biomechanics of two fixation devices at the time of implantation and three weeks later; this study served as a model for the present study. In that study, Singhatat et al. [9] demonstrated that the biomechanical properties of a complex measured after implantation are not the same as those measured at implantation and may either deteriorate or improve, depending on the type of fixation, either distant from the joint entrance (suspended fixation) or near the tunnel entrance (aperture or anatomical fixation). Among the devices tested by the authors, the WasherLoc (Arthrotek, Warsaw, Indiana, USA), which provided a suspended fixation, maintained its strength and improved its stiffness three weeks after implantation, while the Bio-Interference Screw (Arthrex, Naples, Florida, USA) significantly lost its strength and stiffness after implantation. On the basis of the results of the present study, the Evolgate could provide a very strong aperture fixation even four weeks after implantation, further improving its properties at twelve weeks.

It is of interest to note that while in the tests performed at 4 weeks failure of the complex occurred in 5 of 6 cases due to the pullout of the tendon from the end of the tunnel, in the tests performed at twelve weeks failure occurred in all cases due to tendon midsubstance rupture, suggesting an almost complete maturation of the biological fixation.

Kilicoglu et al. [7], using an ovine patellar tendon, attempted to determine changes in the fixation strength of a poly-L-lactide bioabsorbale interference screw 6 and 12 weeks after impantation. They found a dramatic reduction in strength of the complex at both 6 and 12 weeks, mainly due to rupture of the patellar tendon. A significant decrease of the strength of the graft, as reported by Kilicoglu et al. [7] and by others [15] in previous animal studies, as a result of graft revascularization or maturation, was not observed in the present study.

There are two different types of ligament insertions to bone: direct insertion, as in ACL, and indirect insertion, as in the medial collateral ligament [16, 17]. Direct insertion is similar to bone tendon junction and comprises four different layers: ligament, unmineralized fibrocartilage, mineralized fibrocartilage and bone. Indirect insertion comprises three layers: ligament, Sharpey's fibers and bone. The main factors affecting the type of insertion seem to be strain, site, length and angle of insertion. When a ligament runs parallel to the bone, as in the medial collateral ligament, the insertion is more likely to be indirect, while when the ligament enters the bone quite perpendicularly (as in ACL), the insertion is direct. Inside the tibial tunnel, the fibers of the hamstrings, and in our ovine model the fibers of the extensor digitorum longus, run parallel to the bony walls of the tunnel and an indirect fixation has to be expected. Although based on few samples, our histological findings, in accordance with the findings of Robert et al. [8] who studied samples from human biopsies, seem to confirm the trend toward an indirect fixation of the tendons inside the bone tunnels, if they are securely fixed. At four weeks, the Sharpey's fibers were only glimpsed but at eight weeks they were easily recognizable. We also speculate that at that time the Sharpey's fibers contributed significantly to the stability of the implant. On the contrary, using a bioabsorbable interference screw fixation, Kilicoglu et al. [7] did not find Sharpey-like fibers at either 6 or 12 weeks; instead, the bone tendon interface was formed of three layers: a superficial band of necrotic cellular debris and active granulation tissue, a middle layer of residual tendon, and the deepest new fibrous tissue formation merging with medullary bone tissue.

In conclusion, besides confirming the reliability of the Evolgate as a tibial fixation device for hamstring tendons in ACL reconstruction, this study provides further evidence that strength and stiffness of fixation change significantly during the immediate post-implantation period and may be different for various fixation devices. This reduces the usefulness of in vitro and cadaveric studies for testing the reliability of a device. Those studies should be considered only as preliminary biomechanical reports due to their lack of ability to predict device effectiveness after implantation. Nevertheless, clinical studies remain the best way to determine the efficacy of a fixation device.

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