Tendon Healing in a Bone Tunnel. Part I: Biomechanical Results After Biodegradable Interference Fit Fixation in a Model of Anterior Cruciate Ligament Reconstruction in Sheep

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Purpose: Interference fit fixation of soft-tissue grafts has recently raised strong interest because it allows for anatomic graft fixation that may increase knee stability and graft isometry. Although clinical data show promising results, no data exist on how tendon healing progresses using this fixation. The purpose of the present study was to investigate anterior cruciate ligament (ACL) reconstruction biomechanically using direct tendon-to-bone interference fit fixation with biodegradable interference screws in a sheep model. **Type of Study:** Animal study. **Methods:** Thirty-five mature sheep underwent ACL reconstruction with an autologous Achilles tendon split graft. Grafts were directly fixed with poly-(D,L-lactide) interference screws. Animals were euthanized after 6, 9, 12, 24, and 52 weeks and standard biomechanical evaluations were performed. **Results:** All grafts at time zero failed by pullout from the bone tunnel, whereas grafts at 6 and 9 weeks failed intraligamentously at the screw insertion site. At 24 and 52 weeks, grafts failed by osteocartilaginous avulsion. At 24 weeks, interference screws were macroscopically degraded. At 6 and 9 weeks tensile stress was only 6.8% and 9.6%, respectively, of the graft tissue at time zero. At 52 weeks, tensile stress of the reconstruction equaled 63.8% and 47.3% of the Achilles tendon graft at time zero and the native ACL, respectively. A complete restitution of anterior-posterior drawer displacement was found at 52 weeks compared with the time-zero reconstruction. **Conclusions:** It was found that over the whole healing period the graft fixation proved not to be the weak link of the reconstruction and that direct interference fit fixation withstands loads without motion restriction in the present animal model. The weak link during the early healing stage was the graft at its tunnel entrance site, leading to a critical decrease in mechanical properties. This finding indicates that interference fit fixation of a soft-tissue graft may additionally alter the mechanical properties of the graft in the early remodeling stage because of a possible tissue compromise at the screw insertion site. Although mechanical properties of the graft tissue had not returned to normal at 1 year compared with those at time zero, knee stability had returned to normal at that time. There was no graft pullout after 24 weeks, indicating that screw degradation does not compromise graft fixation. **Key Words:** Anterior cruciate ligament—Soft-tissue graft—Biodegradable interference screws—Biomechanics—Animal model.

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Reconstruction of the anterior cruciate ligament (ACL)-deficient knee using autologous hamstring tendon grafts has recently become more popular.[1-4] This may be related to reduced harvest-site morbidity of hamstring tendons compared with bone–patellar tendon–bone graft and an improvement in graft fixation and corresponding operative techniques within the last few years.[3,5-10]

Currently, hamstring tendon graft fixation is performed extracortically using spiked washers, staples or suture, and tape fixation to a button, an anchor, or a post. The extracortical fixation may be responsible for the inferior stiffness and higher elongation due to the construct's length and the additional stretch-out of suture material.[11-13] This may partially explain the higher incidence of anterior knee laxity in ACL reconstructed knees using hamstring tendons compared with the bone–patellar tendon–bone graft.[3,14]

It has been shown that the level of fixation of an ACL graft has a strong influence on anterior knee stability and graft isometry.[15,16] Therefore, anatomic graft fixation at the level of the original ACL insertion sites is the most preferable.[17-21] Given this argument, it is reasonable to hypothesize that the biomechanical properties of conventional hamstring tendon graft fixation techniques could be improved if anatomic fixation were used.

To facilitate anatomic hamstring tendon or quadriceps tendon graft fixation, recent techniques describe the use of direct tendon fixation with blunt threaded titanium or biodegradable interference screws.[20,22] Although clinical data show promising results[23,24] and biomechanical studies have proven the initial fixation strength of soft-tissue graft interference fit fixation,[12,25-29] there has been no analysis of the progression of tendon healing using this fixation. This information is essential in order to customize postoperative rehabilitation and to evaluate the appropriateness of this new technique. The goal of the present study was to examine biomechanically the healing stages of ACL reconstructions in a sheep model using direct tendon-to-bone interference fit fixation with biodegradable poly-(D,L-lactide) interference screws. Specifically, we asked the following research questions: (1) How does graft maturation progress biomechanically: especially, what changes the failure load and the failure mode? (2) Does screw fixation compromise graft maturation? (3) Does screw degradation compromise graft fixation biomechanically?

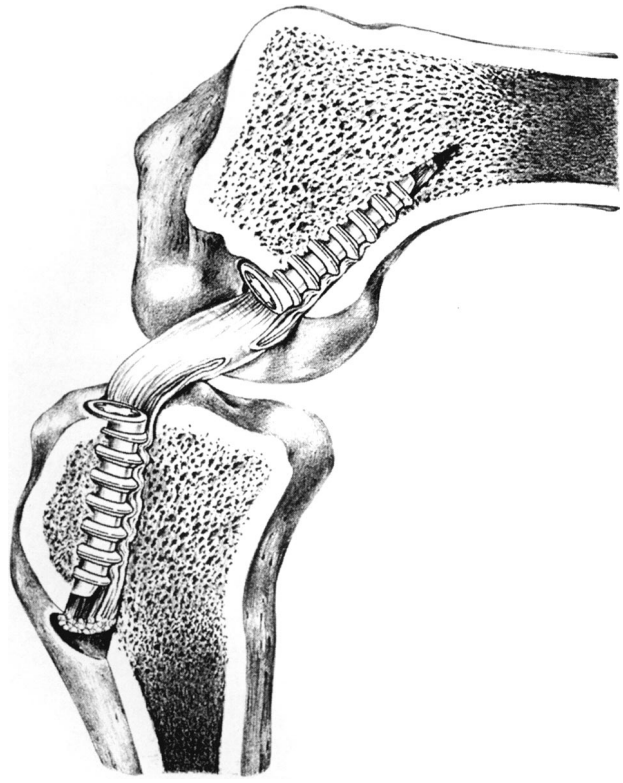

FIGURE 1. Replacement of the ACL with the single-strand Achilles tendon split graft and direct biodegradable poly-(D,L-lactide) interference screw fixation. Screws were inserted in an inside-out direction on the femoral and tibial site.

METHODS

Study Design and Operative Procedure

Thirty-five skeletally mature female merino sheep (mean weight, 51.4 ± 8.7 kg) were used in this study. All animals were screened to ensure that they were in good physical condition. The sheep underwent replacement of the ACL in an open fashion (Fig 1). All procedures were performed with permission of the local governmental animal rights protection authorities in accordance with the National Institute of Health guidelines for the use of laboratory animals.

Five groups with 7 animals each were assigned and animals were euthanized at 6, 9, 12, 24, and 52 weeks. Twelve contralateral knees with intact ACLs served as control. Additionally, 10 contralateral reconstructed knees and 12 Achilles tendon split grafts for time-zero control were tested.

Animals received premedication with intramuscular xylazine hydrochloride 2% and atropine. Anesthesia was then induced intravenously with thiopental-so-

dium. After intubation, anesthesia was maintained with isoflurane and nitrous oxide. All animals received 2.2 g amoxicillin clavulanate intravenously for perioperative antimicrobial prophylaxis. The left hind limb was shaved and prepared in the standard sterile fashion.

A half split graft of the ipsilateral Achilles tendon was used. Through an anterolateral longitudinal skin incision, the calcaneus communis tendon was exposed and the Achilles tendon was carefully separated from the long flexor digitorum tendon. Half of the Achilles tendon, 7.5 cm in length, was sharply dissected and the remaining tendon part was left within the flexor digitorum tendon. The incision was closed in layers using polyglactin and polyamidic sutures. The harvested graft length allowed an interference fit fixation of at least 2.5 cm on both sides, leaving an intra-articular distance of approximately 1.5 cm. Polyester sutures were passed through both ends of the Achilles tendon graft in a modified Bunnell technique, which was then kept moist in saline-soaked gauze.

The knee joint was exposed through an anteromedial incision with release of the medial parapatellar retinaculum.[30] The patella was displaced laterally, the anterior fat pad was sharply separated, and the plica synovialis and the ACL exposed and removed. The ACL insertions sites were cleared of all remaining soft tissue while protecting the posterior cruciate ligament (PCL). With a sharp punch, a small wallplasty of approximately 2 mm was performed at the lateral aspect of the intercondylar notch. A 6-mm tunnel was drilled from inside in an outward direction in 90° of knee flexion at the original tibial insertion site of the ACL. The tunnel was then enlarged by dilation with a 7-mm dilator (Instrument Makar, Okemos, MI) and the cortical bone at the tunnel entrance was then carefully chamfered to a diameter of approximately 8 mm. Using a tunnel notcher (Linvatec, Largo, FL), a small notch was created at the anterolateral aspect of the tunnel along the tunnel wall as a guide for screw insertion to prevent clockwise rotation of the graft around the screw. In maximum knee flexion, the femoral tunnel was created in the 1 o'clock position in the same fashion, leaving approximately 2 mm of the dorsal femur cortex. The femoral tunnel wall was notched at the 12 o'clock position and the graft was then inserted via the holding sutures using a Beath pin.

The biodegradable interference screw consists of pure poly-(D,L-lactide) (Sysorb; Sulzer Orthopedics, Baar, Switzerland). Screw parameters were as follows: length of 23 mm, core diameter of 6.2 mm, and thread diameter of 8 mm. A biodegradable interference screw was chosen because the use of these implants has recently gained strong interest for direct soft-tissue graft fixation[21,31] and because they also allow for uncompromised histologic preparation and evaluation.

Under bilateral graft tensioning, the first interference screw was inserted in the femoral tunnel. The knee was then taken through several full ranges of motion under 90 N of graft pretensioning using a tensiometer (Sulzer Orthopedics). The tibial screw was inserted in an inside-out direction in 90° of flexion under pretension with 90 N (Fig 1). A tibial inside-out screw insertion was chosen because sheep have a very short distance of cancellous bone between the articular surface and the medullary cavity for interference fit fixation. (In cadaver pilot work, an outside-in screw placement resulted in a weak fixation.) The separated anterior fat pad was closed with polyglactin sutures and the medial parapatellar retinaculum was repaired with a strong polyglactin suture. The incision was closed in layers using polyglactin and polyamidic sutures. The animals were then returned to their cages and were allowed to bear full weight with no limitation of range of motion.

After surgery, and for 3 days postoperatively, analgesia was given with an intramuscular injection of tramadol (50 mg) and methimazole (1.25 g). Two weeks after surgery, animals were transferred to an external institution to move without restriction. During the first 14 postoperative days, on a daily basis, and before sacrifice, gait was recorded. Normal gait was defined as no visually detectable abnormalities during running and jumping.

Mechanical Testing

Animals were put down using thiopental-sodium followed by an overdose of potassium chloride. The knees were harvested leaving the joint capsule, the collateral ligaments, the menisci, and the PCL intact. Any visually detectable chondral pathology of the operated and nonoperated knee was recorded separately for the medial, lateral, and patellofemoral compartments. The specimens were wrapped in saline-soaked gauze and were stored at −20°C until testing. Twelve hours before testing, the knees were thawed at 4°C. The tibial and femoral bone ends were cleaned of all remaining soft tissue and were embedded in aluminum cylinders using polymethylmethacrylate. During all preparations and testing, specimens were kept moist with saline spray. All mechanical testing was

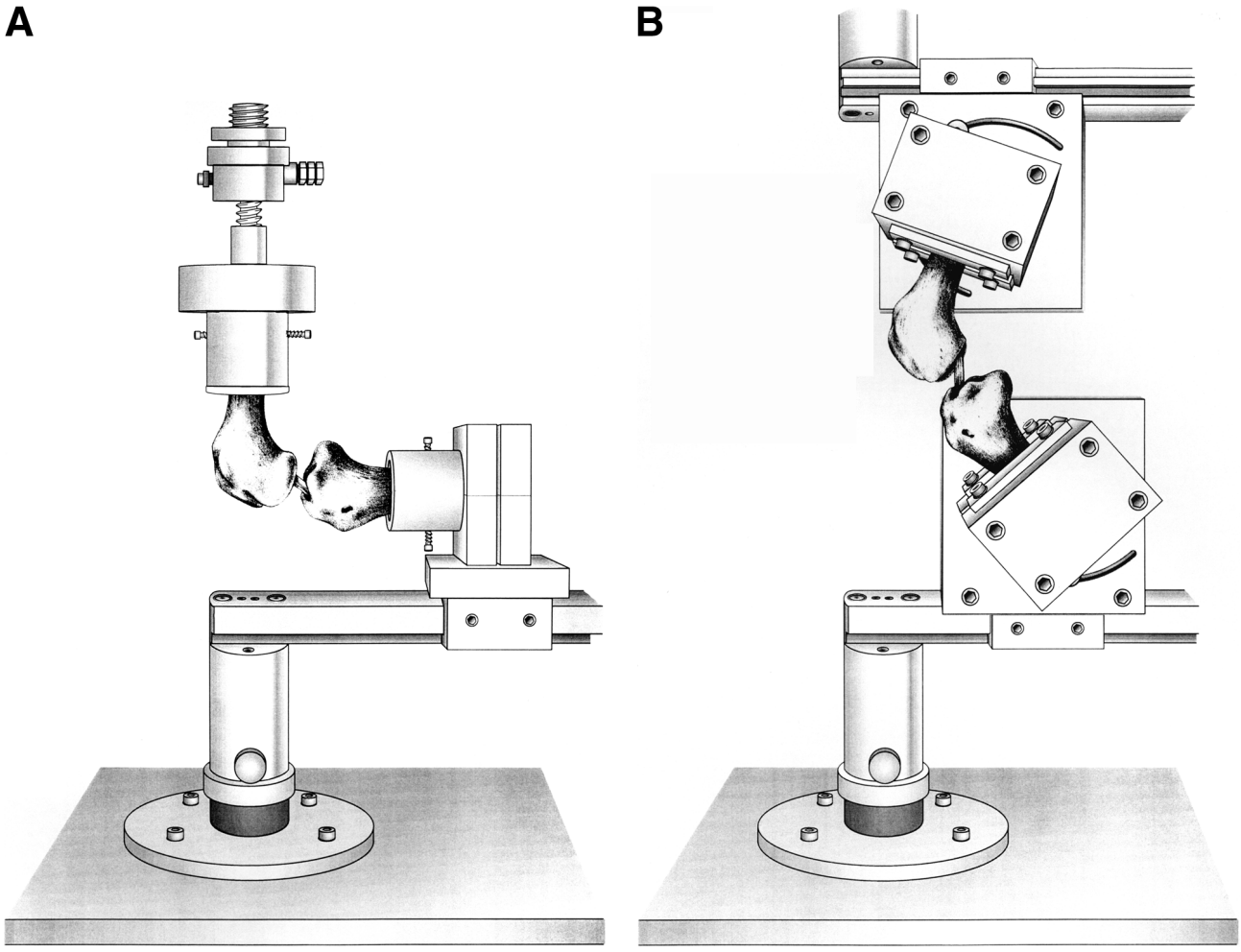

FIGURE 2. (A) The knee mounted in 90° to the materials testing machine for AP drawer testing. (B) The femur-ACL-tibia construct for failure testing with a flexion angle of 60° and the graft aligned parallel to the axis of the applied load. The adjusting device allows free rotation under different flexion angles.

performed according to a consensus recommendation for ACL testing in sheep.[32]

Drawer Tests: The drawer tests were performed in 90° of flexion and neutral rotation with an anterior-posterior femoral load application. The knee was mounted to a material testing machine (Zwick 1455; Zwick GmbH, Ulm, Germany) in a custom-made adjusting device (Fig 2A). To determine the neutral knee position, an anteroposterior (AP) force of 25 N in both directions was applied. The construct was then adjusted in a way that the area of linear laxity in the hysteresis curve was parallel to the x axis. After adjustment, an AP force of ± 50 N was applied 3 times with a load displacement rate of 1 mm/sec. The third hysteresis curve was recorded and transferred to in-house software and the AP displacement was quantified. After AP drawer testing, all soft tissue including the menisci was removed, leaving only the ACL, while the specimen was still mounted to the testing machine. Each specimen was visually checked in the apparatus before testing to ensure that the femoral condyles were congruent with the tibial plateau without any compression force between them, so that the graft was directly exposed to the applied load. The anterior drawer test (50 N, 1 mm/sec) was then repeated and drawer stiffness and absorbed energy were calculated from the hysteresis curve.

Tendon Cross-sectional Area: Following drawer testing, the tendon cross-sectional area was determined according to the technique described by Ellis.[33]

An area micrometer (Mitutoyo, Osaka, Japan) with different sized slots and a force transducer (± 0.1 N) was used. After resecting the medial femoral condyle, each measurement was performed 3 times at the midsubstance portion of the graft at a force of 4.52 N and the average was recorded.

Failure Test: For failure testing, specimens were mounted to a custom made adjusting device which allows free rotation of the construct in different knee flexion angles (Fig 2B). The ACL graft was carefully aligned parallel to the axis of the applied load in 60° of knee flexion. With a preload of 1 N and a load displacement rate of 1 mm / sec specimens were loaded until failure. Maximum load to failure, stiffness, and energy to failure were calculated from the load displacement curve. Failure modes (graft rupture at femoral or tibial insertion site, midsubstance rupture, bony avulsion or femoral or tibial graft pull-out) were recorded.

Failure Test Achilles Tendon Graft: To determine the mechanical properties of the graft at time zero, Achilles tendon split grafts were harvested from the contralateral limb at time of sacrifice in 12 animals. After determining the cross-sectional area, tendons were attached to a cryo-clamping device,[34] leaving a free tendon length of 3 cm between the clamps. The tendon ends were secured with blunt clamps and were deep frozen with dry ice. A preload of 5 N was applied and specimens were then loaded until failure with a displacement rate of 1 mm/sec.

Data Analysis

To determine the absorbed energy during drawer testing and the energy to failure, the load displacement data were entered into in-house software. The hysteresis area and the area below the load displacement curve were determined using Maple V Release 4 software. Maximum load to failure and cross-sectional area data were used to calculate tensile stress.

Data were analyzed for equal distribution using the Kolmogorov-Smirnov test. Because equal distribution testing failed, unpaired comparisons between the control groups and changes between the groups over time were performed using the Mann-Whitney *U* Wilcoxon rank-sum test. The differences were considered to be significant at a probability level of $P \leq .05$. To determine the functional relationship between drawer stiffness and AP drawer displacement, a regression analysis was performed.

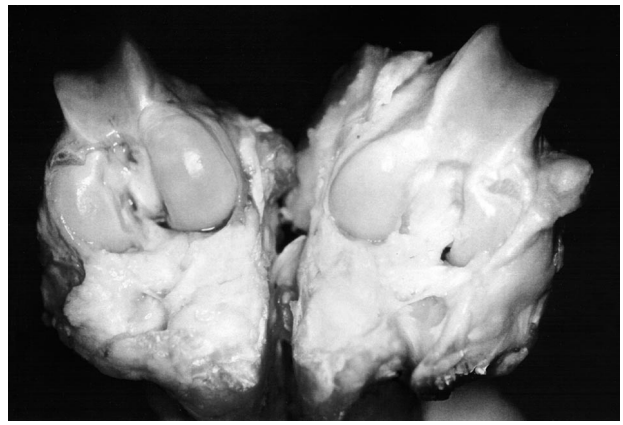

FIGURE 3. Knee joints harvested 52 weeks after ACL reconstruction. Grossly, there is no increased degeneration of the operated left knee (right) and the contralateral nonoperated knee (left).

RESULTS

General

Five animals had to be excluded, 2 because of surgery failure (dorsal femoral wall break-out) and 3 as a result of death not directly related to the surgery (pneumonia, pericarditis, intestinal obstruction) after 5 days, 5 weeks, and 27 weeks. In the 52-weeks group, 1 reconstruction failed. In this specimen, a thin fibrous graft residuum adjacent to the PCL was found. At 6 to 24 weeks, 6 specimens were included in each group and, in the 52-week group, 5 specimens were included into the final evaluation.

All animals partially loaded their left hind limb immediately after surgery and reached normal gait within the first 2 weeks, which was defined as no visually detectable limping during running and jumping. Arthrotomy and tendon-harvest sites healed without any complications in all animals. Before death, all animals displayed normal gait as defined above. Gross inspection of the knee joints at harvest showed that all grafts were covered by an intact synovial membrane with mild inflammatory changes with hypervascularization and thickened synovial tissue. These changes were maximally pronounced at 6 weeks, but could not be detected at all after 12 weeks. No increased chondral or meniscal pathology compared with the contralateral nonoperated knees was found in any of the 3 compartments (Fig 3). After 6, 9, and 12 weeks, the interference screws were still intact, whereas after 24 weeks they were macroscopically degraded, leaving a fibrous capsule at the implant site with a central fluid accumulation.

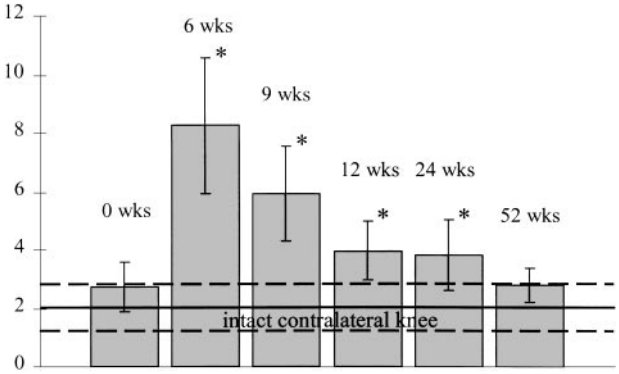

FIGURE 4. Changes of AP drawer displacement (mm) in 90° of flexion at ±50 N. The bars indicate the mean values and the I bars indicate the standard deviations. The horizontal line shows the mean value for AP displacement of the intact contralateral knees and the dotted lines the corresponding standard deviation (*significantly different from the time zero reconstruction). All data were significantly different from the intact contralateral knee.

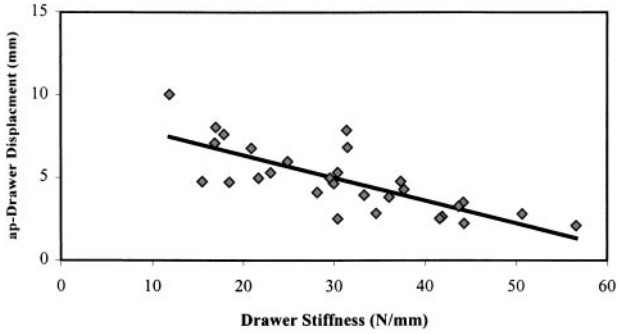

FIGURE 5. Negative linear correlation between drawer stiffness and AP drawer displacement ($R^2 = .583$, $P < .0001$).

Biomechanical Findings

Drawer Testing: At time zero, the average AP drawer displacement was 2.74 ± 0.86 mm, which was significantly different from the intact contralateral knee (2.04 ± 0.69 mm, $P = .03$). The mean AP drawer displacement increased dramatically to 404.4% (8.25 ± 2.31 mm) compared with that of the intact contralateral knee at 6 weeks and 290.7% (5.93 ± 1.67 mm) at 9 weeks, leaving still a moderate increased mean displacement of 194.6% (3.97 ± 1 mm) and 186.8% (3.81 ± 1.21 mm) at 12 and 24 weeks, respectively. Compared with the time-zero reconstruction, there was no significant difference for AP drawer displacement at 52 weeks (2.78 ± 0.5 mm) (Fig 4).

Only at 6 and 9 weeks were drawer-stiffness values significantly lower than those of the intact ACL and the time-zero reconstruction (Table 1). Absorbed energy during anterior drawer testing at 52 weeks (4.1 ± 1.2 N/mm^2) was not significantly different from that of the intact ACL (2.6 ± 2.7 N/mm^2), whereas all other values were significantly lower (Table 1). A significantly negative linear correlation between drawer stiffness and AP drawer displacement with $R^2 = .583$ ($P < .0001$) was found over all groups (Fig 5).

Cross-Sectional Area: Cross-sectional area measurements showed no significant difference between the graft of the time-zero reconstruction, the intact ACL, and the graft for time-zero tensile testing (Table 1). Compared with the time-zero Achilles tendon split graft (27.9 ± 4.9 mm^2) the cross-sectional area data

TABLE 1. *Results of Biomechanical Testing, Except Anterior Drawer Displacement and Ultimate Tensile Strength (mean ± SD)*

Time After Operation	Maximum Load to Failure (N)	Cross-Sectional Area (mm^2)	Stiffness at Anterior Drawer (N/mm)	Stiffness at Failure (N/mm)	Absorbed Energy During Anterior Drawer (N/mm^2)	Energy to Failure (N/mm^2)
0 weeks (n = 10)	267 ± 82*†	24.4 ± 3.6	39.4 ± 7.4	41.2 ± 13*	15.3 ± 8.5*	1235.5 ± 257.8*
6 weeks (n = 6)	44.8 ± 4*†‡	18.9 ± 8.6*†	18.3 ± 4.4*‡	14.4 ± 5.5*‡	69.4 ± 26.9*‡	118.7 ± 37.8*‡
9 weeks (n = 6)	105.6 ± 43*†‡	26.5 ± 9.6‡	24.7 ± 7*‡	33.3 ± 14.4*	40.6 ± 19.2*‡	440.3 ± 53*‡
12 weeks (n = 6)	237.8 ± 59.8*†	37.5 ± 7.5†	33.5 ± 7.2	51.2 ± 11.2*	11.4 ± 4.2*	919.2 ± 276.4*‡
24 weeks (n = 6)	313.8 ± 164.4*†	29.5 ± 9.8	35.9 ± 6.4	58.6 ± 25.9*	9.1 ± 3.9*	1133.3 ± 506.9*
52 weeks (n = 5)	684.9 ± 252.8*†‡	27.8 ± 7	45.6 ± 8.4	90.5 ± 30.3*‡	4.1 ± 1.2‡	2509.1 ± 819*‡
ACL (n = 12)	1531.3 ± 180.3†‡	35 ± 1.8	44.5 ± 12.5	143.9 ± 16.1‡	2.6 ± 2.7‡	8592.7 ± 1564.6‡
Achilles tendon graft (n = 12)	1120.1 ± 223.4*‡	27.9 ± 4.9				

*Significantly different from the intact anterior cruciate ligament ($P \leq .05$, Mann-Whitney *U* Wilcoxon rank-sum test).
†Significantly different from the intact Achilles tendon split graft at time zero ($P \leq .05$, Mann-Whitney *U* Wilcoxon rank-sum test).
‡Significantly different from the reconstruction at time zero ($P \leq .05$, Mann-Whitney *U* Wilcoxon rank-sum test).

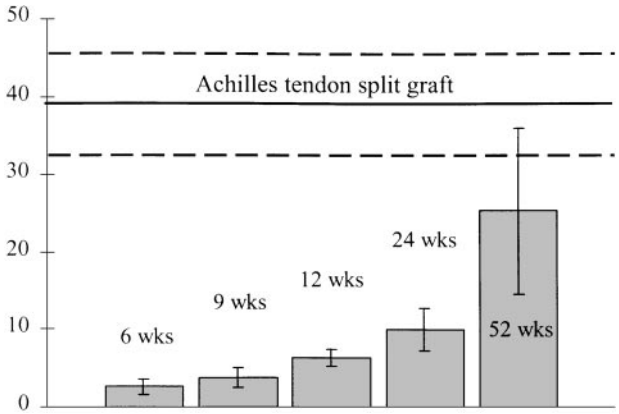

FIGURE 6. Changes of tensile stress (MPa) over time. The bars indicate the mean values and the I bars indicate the standard deviations. The horizontal line shows the mean tensile strength of the intact contralateral Achilles tendon split graft at time zero and the dotted lines the corresponding standard deviation. All data were significantly different from the Achilles tendon split graft at time zero.

indicate a graft atrophy at 6 weeks (18.9 ± 8.6 mm^2, $P = .041$) and a hypertrophy to be present at 12 weeks (37.5 ± 7.5 mm^2, $P = .003$) (Table 1).

Failure Test: All specimens at time zero failed by graft pull-out from the tibial or femoral tunnel, whereas all specimens at 6 and 9 weeks failed at midsubstance. In the 6-weeks group, 2 specimens failed at the midsubstance portion of the graft and 4 near the femoral or tibial insertion sites. At 9 weeks, all grafts failed near the insertion sites. Failure mode after 12, 24, and 52 weeks was osteocartilaginous avulsion at the tibial or femoral insertion site. Intact contralateral ACLs failed either in midsubstance near the insertion sites (n = 8) or by femoral bony avulsion (n = 4).

The mean tensile stress of the Achilles tendon split graft (39.8 ± 7.8 MPa) exhibited 74.1% that of the intact ACL (53.6 ± 13.6 MPa). The maximum load to failure of the time-zero reconstruction (267 ± 82 N) exhibited 17.6% the intact ACL (1513.3 ± 180.3 N). After 52 weeks the mean tensile stress (25.4 ± 10.8 MPa) reached 63.8% and 47.3% that of the Achilles tendon split graft and the intact ACL, respectively, and a maximum load to failure (684.9 ± 252.8 N) of 61.2% and 45.2%, respectively (Table 1), whereas the mean maximum load to failure exhibited 256.5% as opposed to the time-zero reconstruction. At 6 (2.7 ± 0.9 MPa) and 9 (3.8 ± 1.3 MPa) weeks tensile stress reached only 6.8% and 9.6% of the Achilles tendon split graft (Fig 6). All tensile stress and maximum load to failure values between time zero and 52 weeks were significantly different from the Achilles tendon split graft and the intact ACL (Table 1).

All stiffness values between time zero and 52 weeks were significantly lower compared with the intact ACL (Table 1). At 6 weeks (14.4 ± 5.5 N/mm), stiffness of the graft was significantly lower than that of the time-zero femur-graft-tibia complex (41.2 ± 13 N/mm), and at 52 weeks (90.5 ± 30.3 N/mm), stiffness values were significantly higher (Table 1).

Energy to failure data between time zero and 52 weeks were all significantly different from those of the intact ACL. Compared with the time-zero reconstruction (1235.5 ± 257.8 mm^2), data between 6 and 12 weeks were significantly lower, those at 24 weeks (1133.3 ± 506.9 mm^2) were not different, and at 52 weeks, energy to failure was significantly higher (2509.1 ± 819 mm^2) (Table 1).

DISCUSSION

Concerning the progression of graft healing and maturation by means of biomechanical parameters, we found that at all testing points after time zero, the graft fixation did not appear to be the weak link of the reconstruction and that direct interference fit fixation with biodegradable interference screws withstands full loads and unrestricted motion in the present animal model. The weak link during the early healing stages was the graft close to its insertion site, leading to a significant decrease of maximum load to failure and construct stiffness at 6 to 9 weeks. However, the maximum load to failure, drawer stiffness, and stiffness at failure of the construct were already restored at 12 weeks compared with the time-zero reconstruction. At 1 year, tensile stress exhibited 63.8% that of the Achilles tendon graft at time zero and 47.3% that of the intact ACL, which is consistent with previous studies using sheep or goats.[35-38]

Several studies have previously investigated cruciate ligament autograft and allograft maturation and reported a decrease in stress and variation in elongation of the graft over the first 9 weeks with some slight improvement in mechanical properties at 12 weeks.[35,36,38-46] In the present study, a severe decrease of tensile stress to 6.8% and 9.6% at 6 and 9 weeks of the Achilles tendon graft at time zero was found. Although a direct comparison between different studies is difficult because of variations in methodology, this observation differs markedly from those previously reported in goats and sheep.[35-40,46-48]

Regarding whether interference screw fixation compromises graft maturation, we found that failure of the

graft at 6 and 9 weeks occurred mainly at the screw insertion site by intraligamentous rupture at the articular tunnel entrance. This may indicate that direct interference fit fixation of a soft-tissue graft additionally alters the mechanical properties during its early remodeling. However, it is still unclear whether this is caused by tissue laceration during screw insertion or by possible cumulative tissue damage at the tunnel edge under cyclical loading of daily activities. At 12 weeks, we found a change in the failure mode from intraligamentous rupture to osteocartilaginous avulsion at the tunnel entrance site. This may be responsible for the significant increase of tensile stress at 12 weeks compared with 6 and 9 weeks. This finding is in contrast to a recent report by Ng et al.[38] They used a bone–patellar tendon–bone graft for ACL reconstruction in sheep and found intraligamentous failures for up to 3 years, whereas their control specimens failed by osseous avulsion. Thus they concluded that the midsubstance portion of the graft represents the weak link even after final graft maturation if a patellar tendon graft is used.[49] In a separate study, using the identical model as presented here, Förster et al. used an extra-articular fixation of the Achilles tendon split graft and found significantly higher maximum load to failure at 6 weeks compared with the present data (Förster et al., unpublished data). This finding suggests that interference screw fixation of soft-tissue grafts may additionally alter the mechanical properties at the tunnel edge during graft maturation. However, a recent clinical study found no increased graft failure with interference fit fixation of hamstring tendons compared with the patellar tendon graft.[23] In humans, interference screws for soft-tissue graft fixation are chosen in relation to the graft cross-section. In the present model, 8-mm screws were used to fix a 4- to 5-mm graft in very dense subchondral bone, thus creating a high insertion torque that may lead to tissue laceration and may not be found in this degree in humans[29]

Holden et al.[50] found that the ACL in goats is loaded up to 150 N during trotting. Although we used sheep in the present study, it is reasonable to hypothesize that the load in the intact ACL exceeded the graft's mean maximum load to failure at 6 weeks. In the present study, only 1 of 35 reconstructions failed and no extreme laxity was found at 24 to 52 weeks, which is in contrast to the 1-year results of Ng et al.[38] They experienced a dramatically increased anterior drawer at 25 N of approximately 500% at 1 year compared with their time-zero reconstruction. In that study, however, a drawer displacement at 3-year follow-up was found that was not significantly different from the intact contralateral knees. At 52 weeks, we found an AP drawer displacement that was not significantly different compared with the reconstruction at time zero. Ng et al. further described extensive articular cartilage degeneration in some specimens at 1 and 3 years that may account for insufficient function of the ACL graft. In the present study, no such changes were found compared with the uninvolved right knees. It is still unclear why the marked decrease in failure load within the first 9 weeks did not lead to a persistent graft lengthening and laxity increase in the present study. The continuous decrease in AP drawer displacement may be due to the improvement in graft stiffness as the linear regression analysis shows (Fig 5). A similar correlation has been found between AP translation and graft cross-sectional area in a goat and monkey model. In that study, Grood et al.[51] hypothesized that an increased anterior drawer reflects a slack graft that is stress shielded, thereby inducing a small cross-sectional area. Decreased stiffness produces a higher displacement per applied load; thus, in the present study, the correlation between graft stiffness and AP drawer displacement presents the changes of mechanical properties over time. Additionally, we found no decreased cross-sectional area at 24 and 52 weeks compared with the time-zero control, which further supports our believe that the ACL graft in the present animal model was well functioning and not stress shielded.

Many open questions remain about the return to vigorous activities after ACL reconstruction in humans. It has been stated by some authors that the severe loss of mechanical properties after ACL replacement in laboratory animals may not be found in humans because cruciate ligament replacement in animals may not be as accurate as in humans because of improper orientation or tensioning. Jackson et al.[52] investigated changes of mechanical properties in an ACL in situ freezing model and found no difference in tensile strength at 6 and 26 weeks compared with the untreated control. They concluded that loss in strength may not be the natural result of the revascularization and healing process and other studies have proven the detrimental effect of improper graft orientation and tensioning on the tissue's mechanical properties.[53-56]

The bony incorporation of a soft-tissue graft in the bone tunnel is the basic requirement for the long-term survival of the soft-tissue graft. Several studies have investigated the tendon-to-bone healing of soft-tissue grafts biomechanically.[57-60] Different intra-articular and extra-articular animal

models have been studied and, thus, different time frames of a proper tendon-to-bone healing have been described. A major limitation in all these studies, as well as in the present one, is the early strength loss of the graft during the remodeling process, leading to midsubstance failure at a certain time. To our knowledge, only 1 study has investigated the tendon-to-bone healing in a bone tunnel biomechanically in larger animals over time. Rodeo et al.[59] studied the healing of a distally attached tendon transfer in an extra-articular model in dogs. They reported that from 2 to 8 weeks the graft failed by pullout from the tunnel. In the present study, only the time-zero specimen failed by pullout. However, different fixation techniques were used and a different tensile strength at the early healing stages was found. Rodeo et al. recommended that the healing ligament should be protected for at least 8 weeks after the reconstruction.[59]

Concerning whether screw degradation compromises graft fixation, we found that the implanted poly-(D,L-lactide) interference screw was macroscopically degraded after 24 weeks. Between 12 and 24 weeks, there was no change in failure mode or a decrease in maximum load to failure, indicating that screw degradation does not compromise graft fixation. Gross inspection showed that, after 6 to 9 weeks, the screw was separated from the intra-articular environment by tissue overgrowth. Therefore, we believe that if a certain osseous graft anchorage has been developed at the joint surface, the screw and the intra-tunnel tendon-bone junction is of less importance for maintaining graft fixation. Commonly used biodegradable interference screws consist of high–molecular weight poly-L-lactide, which is known to show no degradation within an appropriate time.[31,61-64] However, to judge the influence of material degradation on graft fixation, a material is required that shows complete degradation within the observation period. The chosen material poly-(D,L-lactide) showed its maximum degradation at 24 weeks and may thus allow for the statement that screw degradation does not compromise graft fixation.

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REFERENCES

1. Brown C, Steiner M, Carlson E. The use of hamstring tendons for anterior cruciate ligament reconstruction. Technique and results. *Clin Sports Med* 1993;12:723-756.
2. Howell S, Taylor M. Brace-free rehabilitation with early return to activity for knees reconstructed with double-looped semitendinosus and gracilis graft. *J Bone Joint Surg Am* 1996;78: 814-819.
3. Aglietti P, Buzzi R, Zaccherotti G, De Biase P. Patellar tendon versus doubled semitendinosus and gracilis tendons for anterior cruciate ligament reconstruction. *Am J Sports Med* 1994; 22:211-217.
4. Rosenberg J, Deffner K. ACL reconstruction: Semitendinosus tendon is the graft of choice. *Orthopedics* 1997;20:396-398.
5. Strobel M. Vorderes kreuzband. In: Strobel M, ed. *Arthroskopische chirurgie*. Berlin: Springer, 1998;361-463.
6. Steiner M, Hecker A, Brown C, Hayes W. Anterior cruciate ligament graft fixation. Comparison of hamstring and patellar tendon grafts. *Am J Sports Med* 1994;22:240-246.
7. Rowden N, Sher D, Rogers G, Schindhelm K. Anterior cruciate ligament graft fixation—Initial comparison of patellar tendon and semitendinosus autografts in young fresh cadavers. *Am J Sports Med* 1997;25:472-478.
8. Maeda A, Shino K, Horibe S, Nakata K, Buccafusca G. Anterior cruciate ligament reconstruction with multistranded autogenous semitendinosus tendon. *Am J Sports Med* 1996;24: 504-509.
9. Yasuda K, Tsujino J, Ohkoshi Y, Tanabe Y, Kaneda K. Graft site morbidity with autogenous semitendinosus and gracilis tendons. *Am J Sports Med* 1995;23:706-714.
10. Weiler A, Scheffler S, Südkamp NP. Aktuelle Aspekte in der Verankerung von Hamstringsehnen—Transplantaten in der Kreuzbandchirurgie. *Chirurg* 2000;71:1034-1044.
11. Höher J, Livesay G, Ma C, Withrow J, Fu F, Woo S. Hamstring graft motion in the femoral tunnel when using titanium button/polyester tape fixation. *Knee Surg Sports Traumatol Arthrosc* 1999;7:215-219.
12. Weiler A, Scheffler S, Göckenjan A, Südkamp N, Hoffmann R. Different hamstring tendon graft fixation techniques under incremental cyclic loading conditions. *Arthroscopy* 1998;14: 425-426.
13. Höher J, Scheffler S, Withrow J, Livesay G, Debski R, Fu F, Woo S. Mechanical behavior of two hamstring graft constructs for reconstrcution of the anterior cruciate ligament. *J Orthop Res* 2000;18:456-461.
14. Johnson D, Houle J, Almazan A. Comparison of intraoperative AP translation of two different modes of fixation of the grafts used in ACL reconstruction. *Arthroscopy* 1998;14:425 (abstr).
15. Ishibashi Y, Rudy T, Livesay G, Stone J, Fu F, Woo S. The effect of anterior cruciate ligament graft fixation site at the tibia on knee stability: Evaluation using a robotic testing system. *Arthroscopy* 1997;13:177-182.
16. Morgan C, Kalmam V, Grawl D. Isometry testing for anterior cruciate ligament reconstruction revisited. *Arthroscopy* 1995; 11:647-659.
17. Palmeri M, Morgan C. The all-inside anterior cruciate ligament reconstruction: A double socket approach. *Oper Tech Orthop* 1996;6:161-176.
18. Scranton P, Pinczewski L, Auld M, Khalfayan E. Outpatient endoscopic quadruple hamstring anterior cruciate ligament reconstruction. *Oper Tech Orthop* 1996;6:177-180.
19. Dye S. The future of anterior cruciate ligament restoration. *Clin Orthop* 1996;325:130-139.
20. Stähelin A, Weiler A. All-inside anterior cruciate ligament reconstruction using semitendinosus tendon and soft threaded biodegradable interference screw fixation. *Arthroscopy* 1997; 13:773-779.

21. Brand J, Weiler A, Caborn D, Brown C, Johnson D. Current concepts: Graft fixation in cruciate ligament surgery. *Am J Sports Med* 2000;28:761-774.
22. Pinczewski L. The use of RCI screw for endoscopic fixation of quadruple strand hamstring tendon autograft in anterior cruciate ligament reconstruction. Presented at the Annual Meeting of the American Academy of Orthopaedic Surgery, AANA Speciality Day, 1997; Book of Abstracts; 202-206.
23. Corry I, Webb J, Clingeleffer A, Pinczewski L. Arthroscopic reconstruction of the anterior cruciate ligament: A comparison of patellar tendon autograft and four-strand hamstring tendon autograft. *Am J Sports Med* 1999;27:444-454.
24. Musgrove T, Salmon L, Burt C, Pinczewski L. The influence of reversed-thread screw femoral fixation on laxity measurements after anterior cruciate ligament reconstruction with hamstring tendons. *Am J Sports Med* 2000;28:695-699.
25. Weiler A, Hoffmann R, Stähelin A, Bail H, Siepe C, Südkamp N. Hamstring tendon fixation using interference screws—A biomechanical study in calf tibial bone. *Arthroscopy* 1998;14: 29-37.
26. Caborn D, Coen M, Neef R, Hamilton D, Nyland J, Johnson D. Quadrupled semitendinosus-gracilis autograft fixation in the femoral tunnel: Comparison between metal and a bioabsorbable interference screw. *Arthroscopy* 1998;14:241-245.
27. Brand J, Steenlage E, Coen M, Caborn D, Johnson D. Interference screw fixation of a quadrupled hamstring tendon is directly correlated to bone mineral density measured by dual photon absorbtiometry (DEXA). Presented at the 2nd Congress of the International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine, Washington, DC, 1999.
28. McKeon B, Donahue B, Tarinelli D, Fulkerson J, Dalsky G. Biomechanical evaluation of a soft tissue interference screw in free tendon ACL graft fixation. Presented at the 45th Annual Meeting of the Orthopaedic Research Society, Anaheim, CA, 1999;284.
29. Weiler A, Hoffmann R, Siepe C, Kolbeck S, Südkamp NP. The influence of screw geometry on hamstring tendon interference fit fixation. *Am J Sports Med* 2000;28:356-359.
30. Allen M, Houlton J, Adams S, Rushton N. The surgical anatomy of the stifle joint in sheep. *Vet Surg* 1998;27:596-605.
31. Weiler A, Hoffmann R, Stähelin A, Helling H, Südkamp NP. Current concepts: Biodegradable implants in sports medicine—The biological base. *Arthroscopy* 2000;16:305-321.
32. Claes L. Empfehlung für tierexperimentelle Untersuchungen zum Bandersatz. *Hefte zu der Unfallchirurg* 1994;234:206-210.
33. Ellis D. Cross-sectional area measurements for tendon specimens: A comparison of several methods. *J Biomechanics* 1969;2:175-186.
34. Hamner D, Brown C, Steiner M, Hecker A, Hayes W. Hamstring tendon grafts for reconstruction of the anterior cruciate ligament: Biomechanical evaluation of the use of multiple strands and tensioning techniques. *J Bone Joint Surg Am* 1999;81:549-557.
35. Amendola A, Fowler P. Allograft anterior cruciate ligament reconstruction in a sheep model. *Am J Sports Med* 1992;20: 336-345.
36. Kasperczyk W, Bosch U, Oestern H, Tscherne H. Staging of patellar tendon autograft healing after posterior cruciate ligament reconstruction. A biomechanical and histological study in a sheep model. *Clin Orthop* 1993;286:271-282.
37. Drez D, DeLee J, Holden J, Arnoczky S, Noyes F, Roberts TS. Anterior cruciate ligament reconstruction using bone–patellar tendon–bone allografts. A biological and biomechanical evaluation in goats. *Am J Sports Med* 1991;19:256-263.
38. Ng G, Oakes B, Deacon O, McLean I, Lampard D. Biomechanics of patellar tendon autograft for reconstruction of the anterior cruciate ligament in the goat: Three-year study. *J Orthop Res* 1995;13:602-608.
39. Bosch U, Kasperczyk W. Healing of the patellar tendon autograft after posterior cruciate ligament reconstruction: A process of ligamentization? An experimental study in a sheep model. *Am J Sports Med* 1992;20:558-565.
40. Jackson D, Grood E, Arnoczky S, Butler D, Simon T. Cruciate reconstruction using freeze dried anterior cruciate ligament allograft and a ligament augmentation device (LAD): An experimental study in a goat model. *Am J Sports Med* 1987;15: 528-538.
41. Butler D, Hulse D, Kay M, Grood E, Shires P, DÂmbrosia R, Shoji H. Biomechanics of cranial cruciate ligament reconstruction in the dog. II. Mechanical properties. *Vet Surg* 1983;12: 113-118.
42. Clancy W, Narechania R, Rosenberg T, Gmeiner J, Wisnefke D, Lange T. Anterior and posterior cruciate ligament reconstruction in rhesus monkeys. *J Bone Joint Surg Am* 1981;63: 1270-1284.
43. Butler D, Grood E, Noyes F, Olmstead M, Hohn R, Arnoczyk S, Siegel M. Mechanical properties of primate vascularized vs. nonvascularized patellar tendon grafts: Changes over time. *J Orthop Res* 1989;7:68-79.
44. McFarland E, Morrey B, An K, Wood M. The relationship of vascularity and water content to tensile strength in patellar tendon replacement of the anterior cruciate ligament in dogs. *Am J Sports Med* 1986;14:436-448.
45. Holden J, Grood E, Butler D, Noyes F, Medenhall H, Van Kamper C, Neidich R. Biomechanics of fascia lata ligament replacements—Early postoperative changes in the goat. *J Orthop Res* 1988;6:639-647.
46. Zimmermann M, Contiliano J, Parsons J, Prewett A, Billotti J. The biomechanics and histopathology of chemically processed patellar tendon allografts for ACL replacement. *Am J Sports Med* 1994;22:378-386.
47. Jackson D, Grood E, Goldstein J, Rosen M, Kurzweil P, Cummings J. A comparison of patellar tendon autograft and allograft used for anterior cruciate ligament reconstruction in the goat model. *Am J Sports Med* 1993;21:176-185.
48. Schindhelm K, Rogers G, Milthorpe B, et al. Autograft and Leeds-Keio reconstruction of the ovine anterior cruciate ligament. *Clin Orthop* 1991;267:278-293.
49. Ng G, Oakes B, Deacon O, McLean I, Eyre D. Long-term study of the biochemistry and biomechanics of anterior cruciate ligament-patellar tendon autografts in goats. *J Orthop Res* 1996;14:851-856.
50. Holden J, Grood E, Korvick D, Cummings J, Butler D, Bylski-Austrow D. In vivo forces in the anterior cruciate ligament—Direct measurements during walking and trotting in quadruped. *J Biomechanics* 1994;27:517-526.
51. Grood E, Walz-Hasselfeld K, Holden J, et al. The correlation between anterior-posterior translation and cross-sectional area of anterior cruciate ligament reconstructions. *J Orthop Res* 1992;10:878-885.
52. Jackson D, Grood E, Cohn B, Arnoczky S, Simon T, Cummings J. The effects of in situ freezing on the anterior cruciate ligament. *J Bone Joint Surg Am* 1991;73:201-213.
53. Keira M, Yasuda K, Yamamoto N, Hayashi K. Mechanical properties of the anterior cruciate ligament chronically relaxed by elevation of the tibial insertion. *J Orthop Res* 1996;14:157-166.
54. Ohno K, Yasuda K, Yamamoto N, Kaneda K, Hayashi K. Effects of complete stress-shielding on the mechanical properties and histology of in situ frozen patellar tendon. *J Orthop Res* 1993;11:592-602.
55. Majima T, Yasuda K, Yamamoto N, Kaneda K, Hayashi K. Deterioration of mechanical properties of the autograft in controlled stress-shielded augmentation procedures. *Am J Sports Med* 1994;22:821-829.

56. Bush-Joseph C, Cummings J, Buseck M, Bylski-Austrow D, Butler D, Noyes F, Grood E. Effect of tibial attachment location on the healing of the ACL freeze model. *J Orthop Res* 1996;14:534-541.
57. Grana WA, Egle DM, Mahnken R, Goodhart CW. An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. *Am J Sports Med* 1994;22:344-351.
58. Blickenstaff K, Grana W, Egle D. Analysis of a semitendinosus autograft in a rabbit model. *Am J Sports Med* 1997;25: 554-559.
59. Rodeo S, Arnoczky S, Torzilli P, Hidaka C, Warren R. Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. *J Bone Joint Surg Am* 1993;75:1795-1803.
60. Yamazaki S, Yasuda K, Tomita F, Mikami, Tohyama H, Miyagi N, Kaneda K. The effect of graft-tunnel diameter disparity on healing of the doubled flexor tendon graft within the bone tunnel in anterior cruciate ligament reconstruction. Presented at the 45th Annual Meeting of the Orthopaedic Research Society, Anaheim, CA, 1999;932.
61. Pistner H, Gutwald R, Ordung R, Reuther J, Mühling J. Poly(L-lactide): A long-term degradation study in vivo. Part I, biological results. *Biomaterials* 1993;14:671-677.
62. Weiler A, Windhagen H, Raschke M, Laumeyer A, Hoffmann R. Biodegradable interference screw fixation exhibits pullout force and stiffness similar to titanium screws. *Am J Sports Med* 1998;26:119-128.
63. Stähelin A, Weiler A, Rüfenacht H, Hoffmann R, Geissmann A, Feinstein R. Clinical degradation and biocompatibility of different bioabsorbable interference screws: A report of six cases. *Arthroscopy* 1997;13:238-244.
64. Bergsma E, de Bruijn W, Rozema F, Bos R, Boering G. Late degradation tissue response to poly(L-lactide) bone plates and screws. *Biomaterials* 1995;16:25-31.