Histological Evaluation of Regenerated Semitendinosus Tendon a Minimum of 6 Years After Harvest for Anterior Cruciate Ligament Reconstruction

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Background: Semitendinosus (ST) and/or gracilis (G) autografts are the most used grafts for anterior cruciate ligament (ACL) surgery. The tendons have been shown to be able to regenerate but with focal areas of scar tissue in the short term. There are no long-term histological studies of the regenerated tendons.

Hypothesis: In the long term, the regenerated ST tendon normalizes and has a similar histology as the contralateral nonharvested tendon.

Study Design: Case-control study; Level of evidence, 3.

Methods: Eighteen patients (8 female, 10 male) who underwent ACL surgery using ipsilateral ST/G tendon autografts were included in this study. Percutaneous specimens were obtained from the regenerated ST tendon and the contralateral nonharvested ST tendon under ultrasonographic guidance at a median of 8.4 years (100.5 months; range, 77-129 months) after the harvest procedure. Specimens from the nonoperated side served as controls. The histology and presence of glycosaminoglycans (GAGs) were assessed using a light microscope and a semiquantitative grading system.

Results: Thirty-six biopsies were obtained (2 biopsies from each patient). In 5 biopsies, the amount of tissue was too small to analyze in the light microscope, and 1 patient had been operated on bilaterally and was therefore excluded. In total, 24 biopsies were included in the histological analysis. In overall terms, there were no significant differences between the regenerated and nonharvested ST tendon in terms of fiber structure, cellularity, vascularity, and level of GAGs a minimum 6 years after harvest of the ST tendon. However, 3 of the regenerated tendons displayed a loss of fiber structure.

Conclusion: The ST tendon regenerates and may regain a histological appearance similar to that of the nonharvested contralateral tendon, as seen in this study a median of 8.4 years after harvesting. However, in some tendons, loss of fiber structure was found.

Keywords: ACL; tendon regeneration; biopsy; histology; semitendinosus

Studies using magnetic resonance imaging (MRI), computed tomography, ultrasonography have shown that the semitendinosus (ST) tendon can regenerate after harvest. With increasing numbers of anterior cruciate ligament (ACL) reconstructions, the amount of revision procedures after failed primary reconstructions will increase as well. If total regeneration of the ST tendon really occurs, this might be a potential source for future graft material in conjunction with ACL revision surgery. Studies concerning the histology of the ST tendon appear important since open biopsy specimens obtained in the short term have shown that real tendinous tissue regenerates. However, focal areas of scar tissue and histological changes compared with the healthy ST tendon can also be found. The literature does not provide evidence that the ST tendon returns to normal, and furthermore, there are no studies where the regenerated tendon is compared with the contralateral nonharvested tendon from the same patients. The purpose of the present medium- to long-term study was to investigate whether the regenerated ST tendon has histology similar to that of the contralateral nonharvested tendon from the same patient, in terms of fiber structure, cellularity, vascularity, and content of glycosaminoglycans (GAGs). The hypothesis of the study was that this would be the case.
MATERIALS AND METHODS

Eighteen patients (8 females, 10 males) who underwent ACL reconstruction using ipsilateral ST and gracilis (G) tendon autografts were included in the study. The patients were a subgroup from a previously published study focusing on tendon regeneration as seen on MRI, who agreed to undergo a bilateral biopsy procedure.

The median age at reconstruction was 23 years (range, 17-40 years). Percutaneous specimens were obtained from the regenerated tendon and the contralateral nonharvested healthy ST tendon under ultrasonographic guidance at a median of 8.4 years (100.5 months; range, 77-129 months) after the harvesting procedure. Specimens from the nonharvested side served as controls. In all, 36 biopsies were obtained.

Surgical Technique

At the index operation, the ST/G autograft was harvested through a 3-cm oblique incision over the pes anserinus. The sartorius fascia was incised parallel to the fibers of the fascia just above the thicker and more distally inserted ST tendon. After the vinculae had been cut under visual control, the full lengths of the tendons were harvested with a semiblunt, semicircular open tendon stripper (Acufex; Microsurgical Inc). The femoral bone tunnels were prepared using a standard transstitial or medial portal approach. No harvest site drain was used.

Rehabilitation

All patients were rehabilitated according to the same accelerated protocol used for all patients at the clinic, permitting immediate full weightbearing and full range of motion. No rehabilitation brace was used. Closed-chain exercises were started immediately postoperatively. Terminal extension with an external load other than the weight of the operated leg was not permitted during the first 6 weeks postoperative. Running was permitted after 3 months, and contact sports after 6 months at the earliest. During the rehabilitation period, no sprains or ruptures were registered in the posterior part of the thigh.

Biopsy Procedure

Specimens were obtained from the ST tendon on the operated and nonoperated side of each patient. The biopsy specimens were obtained under ultrasonographic guidance with a free-hand technique using a 1.2-mm Tru-cut Monopry instrument (Bard Inc). This is a metal handle with a preattached disposable biopsy needle. The gun needle moves in 2 stages when fired. During the first stage, the inner stylet punctures the target and, in the second stage, an outer cannula follows the path of the stylet, covering the sample notch and thus capturing the sample. Local anesthesia with adrenaline (5-10 mL) was given subcutaneously. Under ultrasonographic guidance, the ST and G tendons were identified proximally on the thigh and followed to a position approximately 4 cm above the medial joint line with the knee in slight flexion. In this position, the specimens were obtained from the central part of the ST tendon through a small incision. Each specimen was placed separately in a coded tube. The specimens had a depth of approximately 5 mm and a maximum diameter of 1.2 mm.

Clinical Assessment

The Tegner activity level and the Lysholm score were used to assess patient function at follow-up.

Evaluation of Histology Using the Light Microscope

The specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 4 to 5 μm, according to routine procedures. The sections were stained with hematoxylin and eosin to evaluate fiber structure, cellularity, and vascularity, and Alcian blue (pH, 2.5)/periodic acid–Schiff (AB/PAS) for the detection of GAG-rich areas. A pathologist and an orthopaedic surgeon, both with a specific interest in and knowledge of tendon pathology, simultaneously examined the tendon specimens using a light microscope (Leica DMRBE). Both examiners were blinded for whether the specimens came from regenerated or nonharvested ST tendon. The specimens were evaluated using a semiquantitative (non-parametric) grading system for the tendon alterations used in multiple previous studies. Grading was based on a 4-point scoring system (Table 1). Fiber structure, cellularity, vascularity, and level of GAGs were graded after examining the entire section. The number of cells was estimated in a high-power field (HPF) representative of the section.

Statistical Analyses

Median (range) values are presented. The Wilcoxon signed-rank test was used for comparisons between the regenerated and nonoperated ST tendon specimens. A value of $P < .05$ was considered significant. When planning the
study, a difference of 1 unit in the classification of fiber structure between regenerated and nonharvested tendons was expected. The required sample size would then be 10 paired specimens to reach a power of 80%, if the standard deviation is 1 unit for the difference between pairs. To allow for lost and nongradable samples, 18 paired specimens were obtained.

Ethics

The Ethics Committee at the University of Gothenburg approved the study. All patients gave their informed consent.

RESULTS

The patients had a median Tegner activity level of 6 (range, 5-7) and a median Lysholm score of 87 (range, 47-100) at the time for biopsy procedure.

Bilateral biopsy specimens were obtained in all patients (n = 36 specimens). The patients experienced no pain or discomfort during or after the biopsy procedures. One patient had undergone ACL reconstruction on the contralateral side and was therefore excluded. Also, the regenerated ST tendon in 1 patient and the nonharvested tendon specimen in 4 patients contained insufficient amounts of tissue for evaluation. This left 16 specimens from regenerated tendons and 13 specimens from the contralateral side; thus, 12 patients (24 specimens) were available for paired specimen comparison. The semiquantitative scoring system revealed no significant differences for the fiber structure, cellularity, vascularity, and the amount of GAGs between the regenerated and nonharvested contralateral side (Table 2). The fiber structure in both the regenerated and nonharvested tendons was classified as median grade 1. However, of the 16 specimens from the regenerated tendon, 3 were classified as fiber structure grade 3 in focal areas, and in 3, increased levels of GAGs were detected. Furthermore, 5 specimens from the regenerated tendons had >200 cells/HPF.

In the remaining specimens from the regenerated tendon tissue and in all 13 healthy tendon specimens, no areas of grade 3 fiber structure or GAGs could be detected. In terms of cellularity, 2 nonharvested ST tendons had >200 cells/HPF (Figures 1 and 2).

### TABLE 1

| Fiber structure                          | Grade 0 | Grade 1 | Grade 2 | Grade 3 |
|-----------------------------------------|---------|---------|---------|---------|
| Cellularity                             | <100 cells/HPF | 100-199 cells/HPF | 200-299 cells/HPF | >300 cells/HPF |
| Vascularity                             | Vessels run parallel to the collagen fiber bundles in the septa | Slight increase in vessels, including transverse vessels in the tendon tissue | Moderate increase in vessels within the tendon tissue | Markedly increased vascularity with clusters of vessels |
| Glycosaminoglycans                      | No alcianophilia | Slight alcianophilia between the collagen fibers | Moderate increase in alcianophilia | Markedly increased alcianophilia |

*aA semiquantitative, 4-point scoring system*\(^{13}\) was used to evaluate the biopsies. HPF, high-power field.

### TABLE 2

| Fiber structure | Regenerated ST Tendon | Healthy ST Tendon | P Value |
|-----------------|-----------------------|-------------------|---------|
| Cellularity     | 1 (0-3)               | 1 (0-2)           | .20     |
| Vascularity     | 1 (0-3)               | 0 (0-2)           | .11     |
| Glycosaminoglycans | 0.5 (0-2)      | 0 (0-2)           | .36     |
| Missing value   | 0 (0-1)               | 0 (0-0)           | .08     |

*Values are reported as median (range). The specimen from 1 of the regenerated tendons and 4 specimens from the nonoperated side contained insufficient amounts of tissue, and 1 patient had undergone anterior cruciate ligament reconstruction on the contralateral side. This left 12 patients for the paired specimen comparison. ST, semitendinosus.

DISCUSSION

The principal finding of the present study was that the regenerated ST tendon appeared similar to the contralateral nonharvested ST tendon when evaluated histologically at a median 8.4 years after harvesting.

To our knowledge, this is the only study in which specimens from the regenerated ST tendon are compared with contralateral nonharvested tendon specimens from the same patients. Furthermore, this is the largest study of histological analyses of regenerated ST tendons. There are few studies addressing the histological appearance of regenerated ST tendon. Previous studies\(^7,8,23\) and 1 case report\(^31\) on open biopsy specimens support our findings that it is tendinous tissue that regenerates and not just scar tissue. However, the longest time period presented in these studies between harvest and biopsy procedure is less than 2.5 years. Reviewing these articles, the authors\(^22\) state that at 2.5 years postoperative, the maturation process is probably not complete. A long-term study presenting the final histological outcome for the entire regeneration process is therefore of particular interest. Eriksson et al\(^7\) obtained open biopsies from regenerated ST tendons in 5 patients at a median 20 months after harvesting, and they reported that the regenerated tendons showed the features of a healthy tendon, but that focally there were small scar-like areas with more irregularly oriented collagen,
increased fibroblastic proliferation, and capillary formation compared with healthy control tendons.

In the present study, the fiber structure in both the regenerated and nonharvested control tendons generally showed slight separation of and increased waviness in the fibers. However, 3 of the regenerated tendons displayed a grade 3 classification in focal areas in terms of fiber structure. These findings are similar to those reported by Eriksson et al. 7

Sulphated GAGs appear in low concentrations in the healthy patellar tendon, 2,3 while high levels are found in Achilles tendinopathy, 19 patellar tendinosis ("jumper’s knee"), 15 ruptured tendons, 12 and tendons subjected to compression forces. 30 The content of GAGs in the present study was not detectable in most regenerated specimens, which could be regarded as a sign of tendon normalization.

The 4-point scoring system used in the present study was initially developed for evaluating alterations in the patellar tendon, with a score of 0 in all the measured items in the healthy patellar tendon. In the present study, the histological score was slightly higher for the nonharvested ST tendon than for the healthy patellar tendon. However, a different morphologic appearance between the patellar tendon and the ST tendon has been described by Hadjicostas et al. 10 They report increased cellularity as well as a tendency toward increased vascularity in the ST tendon in 20 cadavers, similar to the findings in the present study.

The way the tendon regenerates has not been clarified. Initially, it was suggested that the tendon regenerates in a proximal to distal direction, 6 and Leis et al 16 termed it “the lizard tail phenomenon.” However, the present view is that the tendon matures uniformly along the harvest site. 5,24,25 Using serial ultrasonography, Papandreou et al 25 described an initial hematoma followed by an edema with gradual solidification along the entire harvest site. This theory is supported by a systematic review, 5 and further proof was recently presented using an animal model for tendon regeneration in Achilles tendons. 24 These authors 24 described a similar regeneration and maturation uniformly along the length of the regenerated tendon. Okahashi et al 23 suggested that the surgical method of “stripping” when harvesting the tendons plays an important role in the regenerative process. Synovial cells possess the ability to differentiate when subjected to mechanical stress. Eriksson et al 7 proposed that the hematoma that occupies the harvest defect acts as a scaffold for the subsequent tendon regeneration. Otoshi et al 24 concluded that the hematoma scaffold enhances migration of fibroblast precursor cells from the surrounding peritendinous tissue and tendon sheath when examining the regeneration process after Achilles tendon stripping in a rat model. In the present study, a standard stripping technique was used for harvesting the ST/G tendon in all patients, and no drain was used. It might be that the initial hematoma is important for the regeneration of the tendons. By stripping

Figure 1. Light-microscopic views of (A-C) a specimen obtained from tendon-like repair tissue and (D-F) the contralateral specimen from nonharvested semitendinosus (ST) tendon tissue. The specimens were obtained 7 years after the harvest procedure from a male patient who was 24 years old at the time of reconstruction. Both sides show linear, parallel-oriented collagen fibers. The regenerated tissue (A-C) shows slight separation and deterioration of fibers, the number of cell nuclei is increased, and there is slight blue-stained alcianophilia between the collagen fibers (C). By comparison, in the contralateral ST tendon (D-F), the fibers are packed; the sparse tendon fibroblasts are thin, oblong, and longitudinally oriented in between the fibers; and there is no alcianophilia (F). Hematoxylin and eosin staining; original magnification 100× (A and D), 200× (B and E), and Alcian blue (pH, 2.5)/periodic acid–Schiff (AB/PAS) staining 200× (C and F).
both tendons at surgery, the risk that the tendon from which the specimen was obtained actually was a nonharvested tendon was eliminated.

The regeneration rate for the harvested tendons differs in the literature from $46\%$ to $95\%$. The reason for this is unknown. Differences in the harvesting technique, time from harvest to study, and patient factors such as smoking could influence the regeneration rate. Furthermore, aggressive postoperative rehabilitation could cause the weak regenerated tendon structure to rupture in the first months after harvest. This is supported by Nakamae et al., who reported 2 cases of unsuccessful regeneration of the semitendinosus tendon at 12 months using 3-dimensional computed tomography. These patients had experienced a sudden sharp pain in the posterior aspect of their thighs when their hamstring muscles were subjected to aggressive load shortly after surgery. This raises the question of whether something happened in the early regeneration process to the patients classified as grade 3 for fiber structure. Trauma with a microbreak in the weak tendon that did not result in a hematoma as seen after tendon stripping might be the cause of loss of fiber structure.

The essential question is whether a regenerated ST tendon can be used for ACL revision surgery in the same way as has been reported for the patellar tendon. There is only 1 case report in the literature in which regenerated ST tendon was used for revision surgery. However, information about the size and strength of the tendon, in addition to histological data, is necessary to predict the outcome. In animal models, the biomechanical strength in the regenerated ST tendon and Achilles tendon has been described as being inferior to that of the healthy tendon up to 1 year after harvesting but with a trend toward increasing strength over time. Since ST tendon regeneration is unpredictable in terms of focal scarring and until studies have been conducted with long-term biomechanical testing in humans, it is the opinion of the authors that regenerated ST tendon cannot be recommended for ACL revision surgery.

The strengths of the present study are its long-term design and the paired biopsies from the patients’ regenerated and nonharvested ST tendon, enabling the patient to serve as her or his own control. A limitation of the study is that 5 biopsies contained an insufficient amount of material for analysis. Although this is the largest study in the literature, there is a potential risk that no significant differences were found due to a type II error. Performing the power analyses on the nonparametric primary variable is also a potential weakness. In spite of the nonsignificant difference, some of the ST tendons displayed focal areas of scarring. It is possible that, by obtaining several biopsies from the entire length of the ST tendon, focal scarring would have been found in more of the regenerated ST tendons. There is a small risk that the biopsies were obtained from the G tendon. However, we chose to only include

Figure 2. Light-microscopic views of (A and B) a specimen obtained from tendon-like repair tissue and (C and D) the contralateral specimen from nonharvested semitendinosus (ST) tendon tissue. The specimens were obtained 6.5 years after the harvest procedure from a female patient who was 18 years old at the time of reconstruction. There is parallelism of the collagen fibers seen in both the regenerated and the contralateral ST tendon. In the regenerated tendon (A and B), there is slight separation and waviness of the fibers and sparse, thin, slender fibroblast nuclei in between the fibers. The contralateral ST tendon specimen (C and D) shows a vessel running longitudinally within the view, and the number of well-oriented fibroblast nuclei is increased. Hematoxylin and eosin staining; original magnification 100× (A and C) and 200× (B and D).
patients in whom both the ST and G tendons were harvested. This means that the biopsy was always obtained from a regenerated tendon. A further limitation is that no intra- or interobserver reliability testing has been performed on the score that was used in this study.\textsuperscript{13,17,18,20} However, the original score from which the score used in the present study was developed has been tested for intraobserver reliability with satisfactory agreements for different tendons.\textsuperscript{19} Finally, since no biomechanical tests of the regenerated ST tendon have been performed, for obvious ethical reasons, the true quality of the regenerated tendon is unknown.

CONCLUSION

The ST tendon regenerates and may regain a histological appearance similar to that of the nonharvested contralateral tendon, as seen in this study, a median of 8.4 years after harvesting. However, in some tendons, loss of fiber structure was found. The hypothesis of the study was thus verified.

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