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ORIGINAL PAPER

Semitendinosus tendon regeneration after anterior cruciate ligament reconstruction: can we use it twice?

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Abstract

Purpose It has been demonstrated that the semitendinosus tendon can regenerate after being harvested in its whole length and thickness for anterior cruciate ligament (ACL) reconstruction. Ultrasound studies and guided biopsies of the regenerated tendon have shown compatibility and resembling features of the normal tendon. The question is if this neo-tendon is biologically and functionally adequate for re-use?

Methods Two randomised groups of 150 volunteers were followed up for two years after harvesting the semitendinosus only (25) or the semitendinosus and gracilis tendons (25) in ACL reconstruction. The patients were followed up with clinical and ultrasound examinations, biopsies and histological tests. Surgical exploration was done in three patients for macroscopic verification. The injected arteries of four lower limbs were dissected and the tendon's arterial supplies were examined.

Results Seventy-two percent of the cases showed regeneration of the semitendinosus tendons. The neotendons were inserted mostly below the knee joint (83.3 %) where they had fused with the gracilis tendon, and above the joint (60 %) when the gracilis was harvested as well. The isokinetic strength of the hamstrings and quadriceps was not significantly diminished on the operated side. A macroscopic and histological analysis of the regenerated tendons demonstrates close resemblance to normal anatomy, with focal areas of fibrosis. In one patient the regenerated tendon was used for medial patellofemoral ligament reconstruction.

Conclusion The semitendinosus muscle can recover and the tendon has great potential to regenerate after harvesting for ACL reconstruction. Our data suggest that the regenerated tendons could be used for iterative ligament reconstruction.

Keywords Semitendinosus tendon regeneration · ACL reconstruction · Gracilis tendon · Re-use of "Neo-tendon"

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Introduction

The semitendinosus (ST) and gracillis (G) tendons are commonly used as a replacement graft during the anterior cruciate ligament (ACL) reconstruction [1]. There are many advantages of using the hamstrings tendons, including the ease of harvesting, suitable morphology for use as a graft, lower donor site morbidity, early rehabilitation, and patient satisfaction [2–4].

In most cases, the ST tendon can regenerate after harvesting, showing similar morphology to the native tendon [5–7]. One study evaluated the semitendinosus muscle by magnetic resonance imaging and biopsies from six to 12 months after isolated harvesting. It identified regenerated ST tendons with the insertion reported to be as a conjoined tendon together with the gracilis in nearly anatomical position at the pes anserinus [5, 6]. Another study provides irrefutable structural evidence that the tendon regenerates with close-to-normal tissue [7].

The recovery of the hamstring muscle function and strength after ACL reconstruction with the ST and G tendons has been investigated by several authors. Although some researchers have demonstrated a loss of hamstring muscle strength in such patients, most investigators have found only slight differences between the operated and the controlateral side in the postoperative period. Recovery of the muscle strength after division of the ST and G tendons can be explained by a process of functional regeneration of the tendons or by compensatory hypertrophy of other knee flexors [4, 6, 8–10]. Morphological changes including atrophy and shortening of the ST have been confirmed in patients with ACL reconstruction using the ST tendon [11, 12].

MRI analysis has indicated a surprising potential for the harvested tendons to regenerate, in particular when only the ST, and not the G, has been used for autologous transplant [7]. Both in vitro and in vivo studies have illustrated "intrinsic" and "extrinsic" healing potential of the injured or surgically reconstructed tendon, although some have shown a regenerative process of the full-length tendon [13–15]. One research investigated the arterial supply of the pes anserinus tendons at the tibial attachment, but not the whole length of the tendons [16].

The aim of this study was to acquire more information and morphologically document the regeneration ability of the semitendinosus after whole length and full thickness harvesting. The final question is if the quality of the regenerated tendons which resembled the normal ones would allow their use as a new graft.

Methods

Between 2009 and 2011, 150 patients were prospectively randomised to ACL reconstruction with either an ipsilateral quadrupled ST graft or an ipsilateral doubled ST/G graft. Included were isolated unilateral traumatic ACL ruptures in patients between 18 and 40 years of age within six months of injury. The patients were then selected according to their willingness to participate in the study, and finally two groups of randomised patients were followed up for two years after harvesting ST (25) or ST/G (25) tendons in ACL reconstruction.

A total of 50 patients (35 males, 15 females; mean age±SD, 25±4 years) were included and participated voluntarily in the study. All patients were either recreational or competitive athletes. Patients were followed up with ultrasound imaging within the specified period of time, i.e., preoperatively, and at one, three, six, 12, 18, and 24 months postoperatively. All procedures were performed in accordance with the ethical standards of the Committee on Clinical Studies of the University of Belgrade's Faculty of Medicine, file number 440/V-9, as well as in the Institute "Banjica". The patients were fully informed of the procedures and the purpose of the study, and gave their written consent for participation.

Arthroscopically assisted reconstructions with an autogenous quadrupled ipsilateral ST tendon or doubled ST/G tendon were performed at the Department of Orthopaedics and Traumatology, Sports Medicine Division of The Institute for Orthopaedic Surgery "Banjica" in Belgrade, Serbia. BioTranfix (Arthrex, Inc) was used for proximal fixation, and interference bioresorptive, osteoconductive PGA screws distally (Arthrex, Inc). The postoperative rehabilitation protocol was the standard one recommended by Rosenberg.

The graft was harvested through a three to five centimetre oblique incision over the pes anserinus, medial to the tibial tuberosity. The sartorius aponeurosis was incised along the course of the ST and G tendons in order to visualise the tendons. The accessory insertion (crural fascicles) of the ST tendon was transected before the semi-blunt, semicircular open tendon stripper (Arthrex, Inc) was applied and pushed in a cranial direction with the knee flexed for better visualisation and for preservation of the saphenous nerve. If the G tendon was needed along with the ST, the stripping procedure was repeated. The distal insertion was harvested along with the free tendon graft. The length of each tendon ranged between 27 and 30 cm; the median graft length was eight centimetres and the median diameter was eight millimetres.

Preoperative ultrasound evaluations (Toshiba, linear structure probe, 15 MHz) were conducted in both groups of patients, on both the involved and the noninvolved leg. The patient was prone with knee flexion of 30° and posteromedial aspect of the knee and thigh were investigated. The structure and margins of the regenerated tendon were analysed in the sagittal plane, as well as the insertion site after the ST tendon or ST/G tendons were harvested (below or above the joint line).

Three patients who were subjected to biopsies were informed that the invasive procedure was solely for scientific purposes; the patients received no benefit for participating in any of the studies. Patients who had the biopsy had sustained new trauma after ACL repair with autologous tendineous grafts and were scheduled for a new surgical procedure—in two cases the ACL revision procedure with meniscus and chondral surgery, and in one case the patellar instability was planned to be solved by medial patellofemoral ligament (MPFL) reconstruction (the biopsy procedure is explained further on). An additional biopsy specimen was taken from a harvested ST tendon in another patient who underwent ACL reconstruction with a combined ST and G graft during the same period for histological comparison; this specimen was taken from a part of the tendon that was discarded after preparation of the graft.

A macroscopic inspection was done before the needle biopsies. The topographic anatomy was similar in all three patients with the regenerated tendons and neotendons located dorsally to the gracilis tendon (all examined volunteers were from the group of patients with only an ST tendon autograft and an intact G tendon). The patients could easily contract their hamstrings including the ST tendon when they were asked to do so (the contracted muscles could also be palpated in the proximal part of the thigh).

A tendon biopsy specimen was obtained under local anaesthesia. Patients were placed in the prone position with their knee flexed with resistance to 45°. A biopsy needle used for liver procedures was used under ultrasonographic control (after the regenerated tendon was identified, including its dynamic movement) on the dorsomedial aspect of the thigh approximately two to six centimetres proximal to the knee joint where the ST and G tendons could easily be palpated. A longitudinal specimen was "pinned" out from the periphery of the regenerated tissue supposed to be ST tendon, fixed in formalin, and embedded in paraffin. The ST tendon was palpated in the distal direction to and below the joint line mostly in patients where only ST was harvested, and proximally for ten to 15 cm in both group of patients.

In one case where the neotendon was used for MPFL reconstruction, a biopsy was conducted during surgery after harvesting the regenerated tendon. The specimens were fixed in 10 % neutral-buffered formalin, embedded in paraffin and stained according to standard technique with H&E stain. Standard light microscopy was used to evaluate morphology according to the routine protocols.

We applied the knee evaluation form (adapted with permission from K. Eriksson) to evaluate the hamstrings strength deficit.

Four lower limbs were injected with a 10 % mixture of India ink and gelatin, and fixed in a 4 % formaldehyde solution for three weeks. The arteries of the tendons were dissected, and precise drawings were made.

Results

The results of the ultrasound investigation of the isolated ST tendon regeneration were compared with sonograms of the non-operated leg as a comparative echo-structure of the normal ST tendon (Fig. 1).

Normal ST tendon collagen fibres were well organised with a longitudinal pattern, and clear and regular edges dominate in uniform echogenic structure. The external tendineous layer, the paratenon, looked like hyperechogenic lines on the tendon edges (Fig. 1a).

In the first two weeks after the ST tendon harvesting, haematoma was visible as well as traumatic oedema of soft tissues. Lower echogenicity and non-organised tissue were present.

Irregular hypoechogenic structure was dominant in the region of anatomical tendineous insertion (Fig. 1b) at the donor site one month after the harvesting.

On the third month ultrasound examination, in the tendon region two centimetres above the joint line, we could isolate tissue mass with an echogenic and hypoechogenic signal which was differentiated compared to the previous examinations. Also, the mass was much larger in cross section than a normal tendon.

Six months after reconstruction we recognised more uniform echostructure which resembled the ST tendon. We could note irregularities even though fibrillar organisation had taken place in tendon structure (Fig. 1c).

One year later we identified well-defined borders and uniform echostructure of the regenerated ST tendon at the point two centimetres above the medial joint line, which we could call a neotendon. There was obvious reduction in cross-sectional diameter compared to earlier sonograms attributed to differentiation.

Eighteen months after tendon harvesting the ultrasound picture showed almost the same tendon structure on the operated side as on the non-operated leg, without irregularities and with well-defined borders (Fig. 1d).

We analysed postoperative patients with ultrasonography and summarised them after isolated ST (group A), and ST and

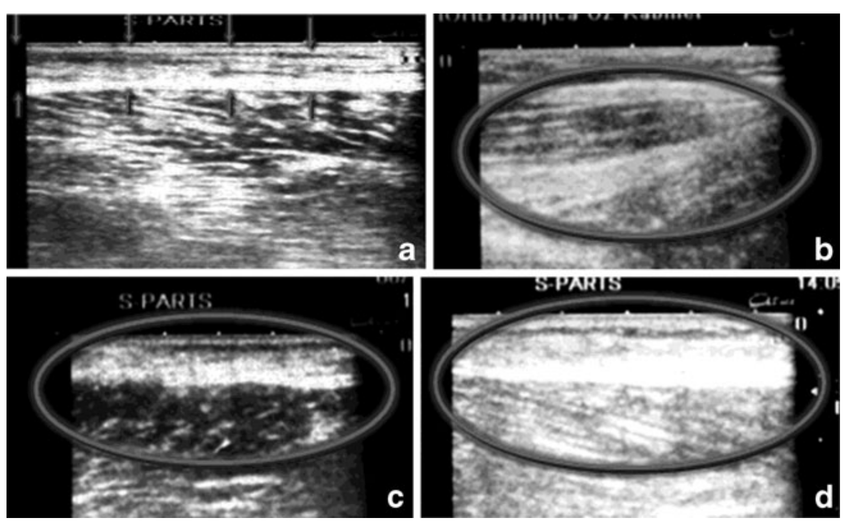

Fig. 1 Longitudinal sonogram of a preoperative semitendinosus (ST) tendon (**a**, outlined by *arrows*), one month after tendon resection (**b**), after six months (**c**), and two years after harvesting (**d**)

G (group B) tendons were harvested for ACL reconstruction (Table 1). The distal insertion area of the regenerated ST tendon was not clearly identified at the anatomical site in the pes anserinus. If only the ST tendon was harvested for autograft, with an intact G tendon, a neotendon developed in 18 (72 %) cases, and the insertion site was mostly below the medial joint line, i.e. in 15 (83.3 %) legs. In cases with a doubled ST/G autograft, the ST neotendon existed in 15 (60 %) legs, and the insertion site was predominantly above the medial joint line on the popliteal fascia, i.e. in nine (60 %) cases.

We evaluated the hamstring strength deficit by applying the knee evaluation form (adapted with permission from K. Eriksson). These results showed no changes in athletic function, and we postulated that the strength of the harvested tendons was not disturbed very much.

The histological appearance of the control ST tendon and the regenerated tendons are illustrated in Fig. 2. The normal ST tendon consisted of parallel bundles of collagen fibres with fibrocytes cells in linear arrangement. The tendon is surrounded by a connective tissue covering (epitendineum), which is continuous with the loose connective tissue between fascicles associated with the small vessels that pass into the interior of the tendon (Fig. 2a).

The regenerated tendon tissue showed the essential features as in the control tendon, with some uniform collagen fibres in a curved (waved) pattern, hypercellularity and blood vessels. Regenerated tendons were with zones of hypervascularisation (neoangiogenesis) and ample blood vessels. Inflammation and necrotic elements were not present as a sign of tissue viability, but focally there were small scar-like areas with more irregularly-oriented collagen (Fig. 2b).

In one patient with traumatic patellar luxation (ACL reconstruction was done three years ago using quadrupled ST graft), the regenerated ST tendon was used for medial patellofemoral ligament reconstruction (Fig. 3). Two years after the follow-up, the patient had no pain, subluxation or dislocation during normal activities.

Table 1 Regenerated tendons and their new insertions in relation to the joint line

Harvested and regenerated tendons	*n* (%)	Insertion site		Total, *n* (%)
		Below joint line, *n* (%)	Above joint line, *n* (%)	
A) ST tendon	25 (100)			
Regenerated	18 (72)	15 (83.3)	3 (16.7)	18 (100)
B) ST and G tendons	25 (100)			
Regenerated	15 (60)	6 (40)	9 (60)	15 (100)

ST semitendinosus

The G tendon was supplied by the small branches which originated from the femoral artery, and from the saphenous branch of the descending genicular artery. Its tibial attaching pes anserinus part had small branches from the inferior medial genicular artery. The ST tendon received one to two branches from the popliteal artery, and from the saphenous artery for its pes anserinus part. The blood supply to tendinous tissue from an arterial network in the epitendineal fibrous covering is sparse, but small arteries from surrounding loose connective tissue and adjacent muscle tissue branching and anastomosing freely, form a complex peritendinous arterial network, a vascular bed of the tendons (Fig. 4).

Discussion

The scheme of tendon regeneration after surgical removal is still unsolved, and we do not really know which of two proposed repair mechanisms, the extrinsic, the intrinsic, or both, is the most realistic [7, 17, 18]. The process of regeneration investigated by ultrasonography in our study and suggested by the sonography findings in the other studies confirmed that fibrillary tendon-like tissue seems to fill the site originally occupied by the ST tendon. Moreover, sonography showed the trend of healing of the donor defect with hypertrophic tendinous tissue seen up to six months after surgery, while the ultrasound structure and measurements are consistent with normal tendon 12 to 18 months after surgery [19].

In one prospective study the incidence of regeneration was found to be 75 %, six to 12 months after harvesting of the tendon [5]. It was also noted that the ST muscle was slightly atrophic, having a smaller cross-sectional area compared to the non-operated side. In a later prospective study where an 83 % incidence of regeneration was found, there were no signs of muscle retraction and histochemistry (fibre type distribution and fibre areas) of the ST muscle specimens, and testing also indicated a functional ST muscle-tendon complex [7]. The study revealed that equal tension in the regenerated ST tendons and adjacent G tendons, when performing a voluntarily hamstring contraction, indicated a functional muscle tendon complex with good mechanical properties. The result rate of 72 % of the ST tendon regeneration revealed regenerated tendon tissue with a normal histological appearance, similar to the control tendon, and the knee evaluation form results showed minimal hamstring strength deficit of the harvested tendon, in agreement with previous studies [7, 15, 16].

In our prospective study, ultrasonography revealed a regenerated and more proximally inserted ST tendon in cases where both tendons were resected, i.e. in 15 (60 %) cases, and then the insertion site was mostly above the joint line on the popliteal fascia, in nine (60 %) of the legs. If only the ST

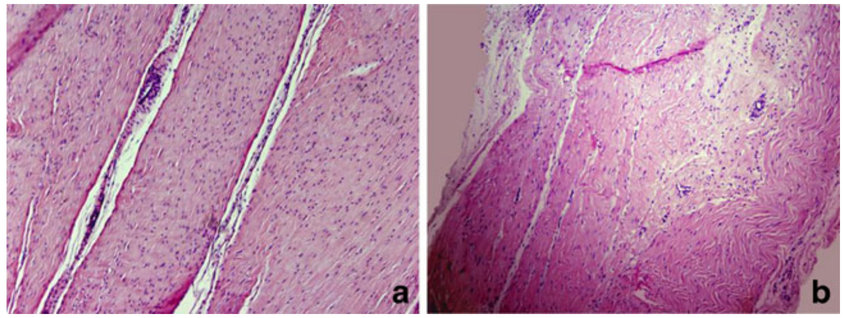

Fig. 2 The histological appearance of the control semitendinosus (ST) tendon (**a**), and the regenerated ST tendon (**b**) (H&E, original magnification x10)

tendon was harvested with an intact G tendon, neotendon developed in 18 (72 %) cases, and then the insertion site was mostly below the joint line but above previous pes anserinus site, in 15 (83.3 %) of the legs. Our ultrasound findings are in agreement with findings from imaging studies in which the regenerated ST tendon seemed to insert more proximally on the gastrocnemius fascia rather than on the tibia, as does the native ST tendon [5, 6, 17–21]. This different insertion pattern of the regenerated tendon could explain how it acts mainly as a flexor muscle rather than as a flexor and internal rotator [22–24].

From our point of view, the vascular bed of a tendon plays the key role in the mechanism of its regeneration. The application of the rounded tendon stripper produces a uniform tubular defect within the fibrous and fatty tissue deep to the fascia lata. A complex arterial network, made of highly interconnected small arteries with origin from the femoral and popliteal fasciocutaneous and musculocutaneous arteries, sends branches to the epitendinum of the harvested tendon. After removal of the whole length tendon, the bleeding from the cut of tiny tendineal arteries and veins stops by persistent ring contractions of the smooth muscles' arterial coat, whereas damaged veins cease to haemorrhage largely by the formation of clots, forming an haematoma in the tendon canal [7, 25].

Fig. 3 Medial patellofemoral ligament (MPFL) reconstruction with regenerated semitendinosus (ST) tendon in a patient with traumatic patellar luxation

The haematoma contains a high concentration of fibrin and platelets, cells producing vasoconstrictor agents, and platelet-derived growth factor (PDGF) which initiates localised inflammation and attracts neutrophils, cells important in releasing vascular endothelial growth factor (VEGF). Vasculogenesis is dependent on VEGF. It is expected that injured vascular capillary networks in the surrounding connective tissue sprout capillary buds along the tubular space of missing tendon. PDGF initiates and guides fibroblast activation, proliferation, and migration [26].

A post-harvesting haematoma might act as a scaffold for mesenchymal stem cells (MSCs) that could invade the area and start fibroblast proliferation and collagen production, and intrinsic healing could be initiated [7]. MSCs with high proliferation capacity, isolated from bone marrow, tendon,

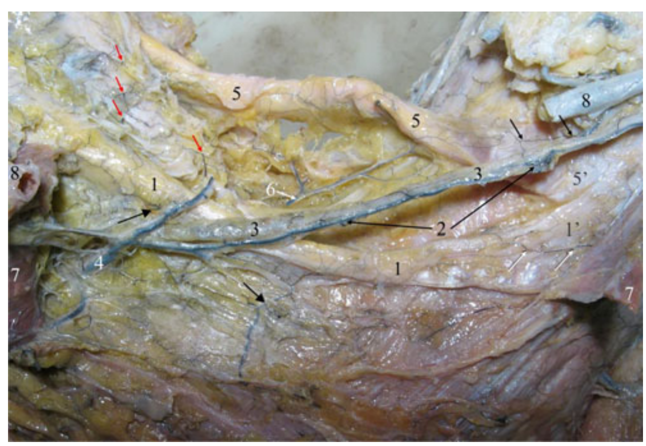

Fig. 4 Medial view of the right knee. G tendon (*1*) is supplied by two small branches (*large arrows*) which originate from the femoral artery, and from the saphenous branch (*2*) (which follows and supplies the saphenous nerve (*3*, elevated) of the descending genicular artery (*4*); its tibial attaching pes anserinus part (*1′*) has small branches (*small white arrows*) from the inferior medial genicular artery. ST tendon (*5*) (retracted) receives large branches (*6*) from the popliteal artery, and from the saphenous artery (*small black arrows*) for its pes anserinus part (*5′*). Small arteries (*small red arrows*), from surrounding loose connective tissue and adjacent muscle tissue, branching and anastomosing freely, form a complex arterial network, a vascular bed of the tendons (arteries injected with India ink and gelatin solution). Sartorius muscle (*7*) is cut and reflected, and great saphenous vein cat and removed (*8*)

epitendineum, and adipose tissue required during regeneration processes, secrete a variety of growth factors and induce angiogenesis. MSCs differentiate into tenocytes (elongated fibroblasts) which are important in collagen synthesis within the extracellular matrix. MSCs produce human foetal tendon-specific matrix components and differentiation factors, which then activate the endogenous regeneration process in tendons [26].

Our anatomical data shows that the vascular bed in the tendon's surrounding tissue is well developed, and could play a crucial role in the regeneration process. We agree that the surgical procedure must be as non-traumatic as possible in order to preserve normal vascular anatomy of the harvesting area and the expected regeneration process [18]. Secondly, our study shows that when the G tendon is left intact, the ST tendon regeneration was more successful in 72 % of cases compared to 60 % when the G tendon was harvested in addition. The explanation could be that the harvesting of the G tendon is the second disturbing intervention within the same vascular territory with possible extra loss of tissue, furthermore, if the G tendon remains, it probably serves as a scaffold for the distal part of the ST neotendon [7].

The lower thirds of the G and ST tendons become flat and fused, covered with sartorius aponeurosis to form pes anserinus, compressed between the crural fascia superficially and the upper part of the medial side of tibia deeply, in a space with little fibrous and adipose tissue present. We noticed an inconspicuous supply of tendons from the saphenous artery, and already described from the inferior medial genicular artery [16]. The surgical procedure with an incision of the sartorius aponeurosis and harvesting of ST tendon distal insertion with the tibial periosteum, completely cuts the supply to the distal part of the area of tendon attachment. If we incise and remove the G tendon as well, which is not only a scaffold for the distal part of the ST neotendon, but the bearer of arterial branches from the inferior medial genicular artery, the whole slit-like attachment area would remain empty, with no other vascular tissue deep to the sartorius aponeurosis. The healing result would be a fibrous closure of the attaching area with no space for any neotendon.

Finally, we should point out that the reharvesting of regenerated hamstring tendons for use as an ACL graft has been reported in literature with an analysis of the ultrastructure of the regenerated tendon [27]. We are of the position that the regenerated tendon might not be strong enough for ACL repair, but after MPFL reconstruction and a morphological and histological analysis in our case, the regenerated ST tendon could be a very good choice for less demanding surgery.

In conclusion we found that the ST muscle can recover, and that the ST tendon has a great potential to regenerate after its removal. Our data showed that those tendons can even be used again for autograft ligamentous reconstruction.

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Conflict of interest None.

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