Higher Rates of Anatomical Insertion of Medial Hamstring Tendon Regeneration Post-Anterior Cruciate Ligament Reconstruction with Stump Preservation Graft Harvesting Technique: A Prospective, Randomized, Double-Blinded Clinical Trial with Magnetic Resonance Imaging Evaluation

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Higher Rates of Anatomical Insertion of Medial Hamstring Tendon Regeneration Post-Anterior Cruciate Ligament Reconstruction with Stump Preservation Graft Harvesting Technique: A Prospective, Randomized, Double-Blinded Clinical Trial with Magnetic Resonance Imaging Evaluation

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The views expressed in this article are of the authors’ own and not an official position of the institution.

Compliance with Ethical Standards

Ethical approval for the study had been obtained from University of Malaya Medical Centre Medical Research Ethics Committee (MREC No: 20161020-4391).

Consent to Participate

Written informed consent has been obtained from all patients prior to enrolment into this study.

Consent to Publish

The Authors hereby consent to publication of the Work in the Journal of ISAKOS

Author Contribution

SHT conceived and designed the experiments, and supervised the project. CAL conducted the experiments and analysed the data. SHT and CAL wrote the manuscript, and jointly developed the structure and arguments for the paper. All authors reviewed and approved of the final manuscript.

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Declaration of Conflicting Interests

There are no potential conflicts of interest with respect to the research, authorship and publication of this article.
Availability of Data and Materials

Research data and materials are available upon request to the corresponding author.

Code Availability

The patient data used in this project were obtained from the Department of Patient Information, UMMC and are strictly confidential.

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Abstract

Objective(s): The purpose of this study was to compare the regeneration of semitendinosus and gracilis tendons from two different graft harvesting techniques which are the stump preservation and conventional graft harvesting technique. We hypothesised that the stump preservation graft harvesting technique which preserved the distal attachment of tendons at their insertion would facilitate anatomical regeneration to the pes anserinus.

Methods: This is a prospective, randomised double-blinded study whereby thirty consecutive patients who underwent single bundle anterior cruciate ligament reconstruction with ipsilateral semitendinosus and gracilis autografts were recruited. The patients were randomly assigned to the stump preservation group (14 patients) or conventional group (16 patients). Magnetic resonance imaging (MRI) evaluation was performed preoperatively and at 6-months postoperatively.

Results: At 6-months follow-up, MRI evaluations showed a higher percentage of insertion of regenerated semitendinosus and gracilis at the pes anserinus in the stump preservation group (75.0%) than that in the conventional group (68.8%). There was significantly higher proximal shift of musculotendinous junction of semitendinosus (5.70 cm versus 3.36 cm, p = 0.029) and gracilis (5.28 cm versus 3.16 cm, p = 0.045) in the conventional group postoperatively.
Conclusion: The stump preservation technique yields higher percentage of anatomical insertion of regenerated tendons and lesser amount of proximal shift of musculotendinous junction.

Level of Evidence: III – Prospective study with up to two negative criteria

Keywords: Anterior cruciate ligament (ACL); gracilis hamstring tendon; magnetic resonance imaging (MRI); pes anserinus harvesting; semitendinosus hamstring tendon; tendon regeneration

(207 words)
What Are The New Findings

- Harvested hamstring tendons for anterior cruciate ligament reconstruction can regenerate
- Shifts in the regenerated hamstring musculotendinous junction could affect contractile strength
- Anatomical insertion was prominent in the group with preserved hamstring tendon stump
- Proximal musculotendinous junction shift was less in stump-preserved hamstring tendon group
Introduction

Arthroscopic anterior cruciate ligament (ACL) reconstruction surgery is becoming an increasingly popular treatment as it allows patients to return to their initial performance level by restoring knee stability. Autografts are widely used among surgeons due to its potential for graft remodelling and incorporation into joints. The benefits of using hamstring autografts are amplified by the ability of semitendinosus and gracilis tendons to regenerate its entire extent. The regenerated tendons have been shown to function as knee flexors and have restored contractile capability.

The biomechanics of the knee are influenced by the extent and insertion of the regenerated tendons. Recent studies found that regenerated tendons tend to have a more proximal insertional point into medial popliteal fossa and tibial plateau when the entire tendon was stripped from its insertion on the tibia. A more proximal insertion causes the tendons’ lever arms to become shorter producing less efficient hamstring contractions leading to postoperative hamstring flexion strength deficit. Another parameter of concern is the proximal shift of the musculotendinous junction of the hamstring muscles which may lead to shortened muscle bellies causing limited contractile strength. Hence, anatomical regeneration of tendons to its tibial insertion at the pes anserinus yielded better postoperative hamstring flexion strength.

The purpose of this study was to compare two different techniques of harvesting semitendinosus and gracilis tendons as autologous grafts for ACL reconstruction, namely the stump preservation and conventional graft harvesting technique. In the conventional technique, the entire attachment of the tendons was removed from the pes anserinus whereas in the stump preservation technique, the distal stumps of the tendons measuring 2 cm were
left attached to the tibia serving as a guide to facilitate regeneration of these tendons to the stumps.

The hypothesis of this study was that the stump preservation technique would yield higher percentage of anatomical regeneration of these tendons to their insertion point at the pes anserinus.

**Methods & Materials**

Ethical approval for the study had been obtained from University of Malaya Medical Centre Medical Research Ethics Committee (MREC No: 20161020-4391). Written and informed consent has been obtained from all participants prior to their enrolment into this study. Study approval was not applicable.

Between April 2017 and April 2018, thirty-four consecutive patients who underwent primary ACL reconstruction using ipsilateral autogenous semitendinosus and gracilis tendons were recruited in the study. Of these patients, four patients were lost to follow-up. The remaining thirty patients were randomly assigned into either the stump preservation group or conventional group. The inclusion criteria include a minimum follow-up duration of six months, harvest of both semitendinosus and gracilis tendons for ACL reconstruction, and absence of hamstring injury in either leg. The exclusion criteria were infection, prior surgery of the affected knee, fractures of either lower extremity in the past, previous hamstring, ACL, or quadriceps surgery, and any contraindications for magnetic resonance imaging (MRI).

**Surgical technique**

In the conventional technique, the semitendinosus and gracilis autografts were harvested through an initial 3 cm incision over the pes anserinus with the knee flexed at 90°. A reverse L-shaped incision was made over the sartorius fascia and the pes anserinus was dissected and
detached along with the periosteum from the tibia (Figure 1A). The tendons were subsequently freed using a closed tendon stripper, after which both semitendinosus and gracilis tendons were folded in half to produce a four-stranded graft. The stump preservation graft harvesting technique utilised the same initial 3 cm skin incision over the pes anserinus. Sartorius fascia was then incised along the superior border of the tendons (Figure 1B). A reverse L-shaped incision was not required as the detachment of the semitendinosus and gracilis insertion was not performed in this method. Instead, the tendons were cut 2 cm proximal to the tibial insertion site, leaving the distal stump attached to the pes anserinus (Figure 1C and 1D). The tendons were then stripped using a closed tendon stripper, after which the distal stump was sutured to the deep portion of sartorial fascia. The same rehabilitative protocol was implemented for patients in both groups. The knee was initially immobilised in a brace at full extension for two weeks. Closed-chain kinetic exercises were started immediately postoperatively. The brace was unlocked by the end of the second week to allow progressive range of motion exercises and muscle strengthening exercises. Partial weight-bearing was permitted 3 weeks after the operation, and full weight-bearing was started after 6 weeks. Patient underwent sports conditioning rehabilitation at 4 months postoperative and sports activities were allowed from 6 to 10 months after the operation. Magnetic resonance imaging (MRI) MRI was performed using a 3-Tesla instrument (Magnetom Prisma, Siemens Healthcare, Erlangen, Germany), and proton density images were obtained in the sagittal and axial planes. The repetition time of all imaging studies was 5580 milliseconds, and the echo time...
was 37 milliseconds. The field of view was 180 x 180 mm, and the matrix size was 400 x 319 pixels. The slice thickness was 3.0 mm for the sagittal and axial planes.

The MRI findings were reviewed retrospectively by an experienced musculoskeletal radiologist and an orthopaedic surgeon, blinded to the preoperative MRI. The MRI images were then randomly selected and repeated in triplicates.

The semitendinosus and gracilis tendons were examined on both axial and sagittal images for the presence of regeneration (Figure 2). Regeneration failure was defined as non-visualisation of tendons on any of the MRI images (e.g., Figure 2B(i)). In cases where regeneration was visualised, the cross-sectional areas of the semitendinosus and gracilis tendons were obtained and calculated at 3.5 cm, 7.0 cm above and at the centre of the medial joint compartment of knee on axial view (see also Supplementary A). The shift in musculotendinous junction was examined in all MRI planes and measured using the distal-most muscle signal intensity of the semitendinosus and gracilis tendons (Figure 3). The insertion site of the tendons was defined as the level measured distally from the centre of medial joint compartment where the two tendons merged. The tendons were traced in the axial dimension using a slice thickness of 0.45 mm with 1-mm distances between the slices to their point of insertion. This was measured using the digital medical imaging system, Picture Archiving and Communication System (PACS), GE Healthcare (Little Chalfont, United Kingdom).

**Statistical analysis**

Prior to commencement of the study, sample size was calculated using an online sample size calculator (https://www.stat.ubc.ca/~rollin/stats/ssize/b2.html), data from published article\(^8\) and to achieve 80% power with Type I error of 5%. The required sample size was 15 patients for each group. The statistical analysis was performed using the SPSS Software version 23.
The distribution of all the variables were determined using Shapiro-Wilk test of Normality, prior to analysis. Descriptive data was expressed as mean ± standard deviation for normally distributed data and median (interquartile range) for skewed data. Frequency and percentage for categorical variables were stated. Independent t-test was used for analysis of normally distributed variables, comparing the mean values between the groups, and paired t-test was used to compare the change of mean pre and post-surgery. Mann-Whitney U test and Wilcoxon signed rank test were used respectively for analysis of non-normally distributed data. A value of \( p < 0.05 \) was statistically significant.

**Results**

There was no statistically significant difference in demographic data between both groups. (age: \( p = 0.725 \); gender: \( p = 0.847 \); height: \( p = 0.482 \); weight: \( p = 0.392 \)).

**Radiologic evaluation**

Patients underwent MRI evaluations preoperatively and at 6 months postoperatively. MRI parameters considered include tendon regeneration, insertion site, cross-sectional area, and magnitude of proximal shift of the musculotendinous junction.

**Number of regenerated tendons**

All 30 patients demonstrated hamstring regeneration after harvest for ACL reconstruction. In the conventional group, semitendinosus regenerated in 15 out of 16 patients (93.8%), while gracilis regenerated in 12 out of 16 (75.0%) patients. On the other hand, the semitendinosus regenerated in 12 out of 14 (85.7%) patients, while gracilis regenerated in all 14 (100.0%) patients in the stump preservation technique group (Table 1).
Insertion site of regenerated tendons

The site of reinsertion was detected in all patients. The insertion of regenerated tendons was found to be at the medial popliteal fascia in five patients (31.2%) from the conventional group and two patients (14.3%) from the stump preservation group. Anatomical regeneration of tendons at pes anserinus was seen in 23 out of 30 patients in which 12 patients were from the stump preservation technique (85.7%) group whereas 11 were from the conventional technique group (68.8%) (Figure 4). Hence, there was higher number of anatomical insertions in the stump preservation group.

Cross-sectional area of regenerated tendons

The mean cross-sectional areas of regenerated tissues in the semitendinosus tendons were 0.25 ± 0.17 cm² in the stump preservation technique group and 0.24 ± 0.12 cm² in the conventional group. Similarly, for the regenerated gracilis tendon, the corresponding values were 0.16 ± 0.11 cm² and 0.15 ± 0.07 cm² in the stump preservation and conventional groups, respectively. Both groups demonstrated increased cross-sectional area postoperatively (Table 2). However, there was no statistically significant difference in the measured cross-sectional area observed between both groups (semitendinosus; \( p = 0.759 \), gracilis; \( p = 0.262 \)).

Proximal shift of musculotendinous junction

Both groups showed proximal migration of the musculotendinous junction in both tendons. The musculotendinous junction of semitendinosus shifted proximally by a mean of 3.36 ± 3.44 cm in the stump preservation group, compared to 5.70 ± 2.40 cm in the conventional group (\( p = 0.038 \)), whereas gracilis migrated proximally by 3.16 ± 2.76 cm in the stump preservation group, compared to 5.28 ± 3.16 cm in the conventional group (\( p = 0.045 \)). This demonstrates a statistically significant higher magnitude of proximal shift of the musculotendinous junction in the conventional group.
At 6 months post-ACL reconstruction, none of the patients from either the conventional
technique group or stump preservation technique group developed complications from the
surgery.

Discussion

Literatures have reported the ability of the native semitendinosus and gracilis tendon to
regenerate after harvest for ACL reconstruction \(^9\text{-}^{11}\). The term “lizard tail phenomenon” was
coined to describe the regeneration of a tendon in a proximal to distal fashion which mimics
the way a lizard regrows its tail \(^12\). The first reported study on regeneration of harvested
semitendinosus and gracilis tendons was published by Cross et al. in year 1992 \(^3\). Subsequent
studies showed that the regenerated tendons possess similar morphological and histological
characteristics to those of the native tendons and were not merely scar tissue \(^4\text{-}^{9,12,13}\).

In our study, we reported regeneration percentage of 85.7\% for the semitendinosus and
100.0\% for the gracilis in the stump preservation group. Literatures have reported
regeneration rates of the semitendinosus and gracilis tendons after harvest for ACL
reconstruction range from 42.7\% to 100\% \(^1\text{-}^{14,16}\). Tadokoro et al. examined 28 patients and
reported 79\% of semitendinosus tendon regeneration and 46\% of gracilis tendon regeneration
\(^17\). A recent systematic review encompassing 18 publications reported mean regeneration rate
more than 1 year after harvesting was 79\% for the semitendinosus and 72\% for the gracilis \(^17\).

The significance of tendon regeneration after harvesting have been shown in the article
published by Nishino et al whereby they found that patients without semitendinosus tendon
regeneration demonstrated lower hamstring flexion strength \(^18\). This finding was attributed to
the change in muscle kinetics owing to the lack of a functional tendon to transmit forces from
the hamstring muscles to the tibia.
The insertion point of the regenerated tendons has always been an area of interest for researchers. In the present study, the regenerated tendons inserted at pes anserinus in 68.8% of the patients in the conventional group and 85.7% of the patients in stump preservation group. Thus, we postulated that the surgical harvesting technique of preserving the semitendinosus and gracilis stumps at the pes anserinus may have increased the percentage of insertion of tendons at their anatomical positions. The stumps of the tendons act as a guide that may help the regenerating tendons track to the pes anserinus and facilitate anatomic reunion. Many studies showed an altered insertional point as the tendons have been found to reattach more proximally. Cross et al. studied four patients who underwent ACL reconstruction and reported that all the regenerated tendons terminated on the medial popliteal fascia at the level of the gastrocnemius. Eriksson et al. reported that the regenerated semitendinosus tendons inserted at the tibial plateau in 8 out of 11 patients postoperatively. It is important for regenerated tendons to achieve anatomical insertion as studies have shown that a more proximal insertion led to decreased knee flexion strength because of shortening of the moment arm.

In our study, the musculotendinous junction of the semitendinosus tendon retracted proximally by average of 3.36 cm in the stump preservation group compared to 5.70 cm in the conventional group, whereas the gracilis migrated proximally by 3.16 cm in the stump preservation group compared to 5.28 cm in the conventional group. This result demonstrates a much lesser proximal shift of musculotendinous junction of semitendinosus and gracilis tendons in the stump preservation group. Using 3-dimensional computed tomography analysis, Nakamae et al. reported that the musculotendinous junction of the semitendinosus tendon shifted proximally by 7.3 cm from the joint line at six months and 7.1 cm at 12 months after surgery in comparison with the contralateral side. Williams et al.
demonstrated that muscle bellies of the semitendinosus and gracilis retract after tendon harvest and remain shortened 6 months after harvest despite tendon regeneration. The reason that all patients had some degree of proximal shift of the musculotendinous junction maybe that there is lack of anchorage to hold these muscles out to length as soon as the tendons are harvested. Choi et al reported a significant correlation between the proximal shift of the musculotendinous junction and flexor deficit in the prone position isokinetic test after hamstring harvesting.

The cross-sectional areas of the regenerated semitendinosus and gracilis tendons were similar to those measured on the preoperative MRI in this study. This is in line with the study by Choi et al. that compared the cross-sectional areas of the regenerated tendons in the preoperative and postoperative MRI and found no significant differences. Tadokoro et al. observed no significant differences when the cross-sectional areas of the regenerated semitendinosus and gracilis tendons of the operated knees were compared with tendons of the non-operated knees. There was no significant correlation shown between cross-sectional area of regenerated tendons and hamstring flexion strength.

The study by Suijkerbuijk et al. on predictive factors of hamstring tendon regeneration after harvest for ACL reconstruction, examined various patient characteristics, including age, sex, body mass index, alcohol and nicotine use. Their findings showed that hamstring tendon regeneration occurs less frequently in older patients and in individuals who smoke. It is important to highlight that, while their investigation investigated into specific predictive factors, our current study did not assess these variables.
Limitations and strengths

The limitation of this study is the relatively short follow-up period of six months. A long-term cohort evaluation may help in further validating the findings of this study. In addition, radiological evaluation of patients at a longer term may also demonstrate delayed regrowth of the harvested tendons. Based on the findings of this study and achieved sample size, power of analysis was found to be between 55.7% and 76.1%. Thus, it is recommended the findings of the study is interpreted with caution.

The strength of this study is the randomised prospective design and proper adherence to the standard follow-up protocol. All patients were subjected to a standard postoperative rehabilitation regime and completed MRI at six-months post-operation. Furthermore, the MRI in this study was performed using a 3-Tesla instrument which allowed a detailed imaging of the regenerated tendons at a higher definition.

Future studies can be performed to evaluate the regenerated tendons and compare their composition to that of native tendons by employing advanced imaging techniques such as MR spectroscopy. By examining parameters such as collagen content and organization, this may reveal the extent to which the regenerated tendons resemble their native counterparts. Furthermore, considering the potential impact of fatty infiltration on long-term outcomes, future studies could be performed to incorporate longer-term follow-ups to assess the degree of fatty infiltration in regenerated tendons. Additionally, to evaluate the functional implications of tendon regeneration, future research should investigate the relationship between semitendinosus and gracilis tendon regeneration on functional performance tests and hamstring flexion strength.
Conclusion

Regeneration of harvested medial hamstring tendons helps to facilitate postoperative strength recovery and limit morbidity. Our study showed that the stump preservation technique yielded higher percentage of anatomical insertion of the regenerated tendons and lesser proximal shift of musculotendinous junction.

Acknowledgement

This study is supported by a private funding grant by Universiti Malaya, UMSC C.A.R.E. (Grant No: PV040-2021).

We would like to thank Professor Dr. John George (Universiti Malaya) and Mr. Mohd Rashdan Abd Rahim (Universiti Malaya) for the assistance and other support in the preparation of this manuscript.
References


**Figure Legend(s)**

**Figure 1.** Diagrams of the incisions made during the hamstring tendons graft harvesting. (A) Conventional technique in which an inverted-L incision was made over the pes anserinus and detached entirely. (B) Stump preservation technique in which an incision was made over the superior border of pes anserinus only. In stump preservation technique, (C) Semitendinosus tendon identified and cut 2 cm from the pes anserinus. (D) Demonstration of the proximal portion of the semitendinosus and gracilis grafts and the ST and G stumps that were retained and sutured to the sartorial fascia. *S: sartorius; *G: gracilis; ST: semitendinosus.

**Figure 2.** Sagittal and axial MRI images of the operated knee, demonstrating (A) the presence of ST and G tendons and (B) the presence of only ST tendon. In both (A) and (B), tendons were visualised through the axial view 7 cm above the centre of the joint line (i), and the tendons were then traced until they merged on the pes anserinus (ii-iv). In (B), the G tendon regeneration was a failure where only the empty tendon sheath can be visualised in the axial view in (B(i)). White arrows indicate the regenerated tendons, while red arrow indicates the empty gracilis tendon sheath with minimal scarring. *G: gracilis; ST: semitendinosus.

**Figure 3.** Sagittal MRI image showing the musculotendinous junction of the regenerated semitendinosus tendon. The regenerated semitendinosus tendon was measured from the joint line.

**Figure 4.** Comparison of insertional site of regenerated tendons in both groups.
Table(s)

Table 1. Regeneration rates of semitendinosus and gracilis tendons according to surgical harvesting technique.

Table 2. Pre- and postoperative cross-sectional area of the semitendinosus and gracilis tendons between the stump preservation and conventional technique groups.
Supplementary Files

Supplementary A. A demonstration of cross-sectional area measurements of tendons, measured at 3.5 cm above the knee joint line.
Table 1. Regeneration rates of semitendinosus and gracilis tendons according to surgical harvesting technique.

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<th>Semitendinosus</th>
<th>Gracilis</th>
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<tr>
<td><strong>Stump preservation group (n = 14)</strong></td>
<td>12 (85.7%)</td>
<td>14 (100.0%)</td>
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<tr>
<td><strong>Conventional group (n = 16)</strong></td>
<td>15 (93.8%)</td>
<td>12 (75.0%)</td>
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Table 2. Pre- and postoperative cross-sectional area\textsuperscript{a} of the semitendinosus and gracilis tendons between the stump preservation and conventional technique groups.

<table>
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<tr>
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<tr>
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<td>Conventional Technique</td>
<td>$p$-value\textsuperscript{d}</td>
<td>Stump Preservation</td>
<td>Conventional Technique</td>
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<tr>
<td>Preoperative CSA \textsuperscript{b} (cm\textsuperscript{2})</td>
<td>0.21 ± 0.05</td>
<td>0.21 ± 0.09</td>
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<tr>
<td>Postoperative CSA \textsuperscript{b} (cm\textsuperscript{2})</td>
<td>0.25 ± 0.17</td>
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<td>$p$-value\textsuperscript{c}</td>
<td>0.456</td>
<td>0.307</td>
<td>0.282</td>
<td>0.487</td>
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</table>

\textsuperscript{a}Results are expressed in mean ± standard deviation

\textsuperscript{b}CSA – cross sectional area

\textsuperscript{c}when CSAs were compared between pre- and postoperative

\textsuperscript{d}when postoperative CSAs were compared between stump preservation technique and conventional technique
Semitendinosus muscle belly

Muscle-tendon junction

Semitendinosus tendon