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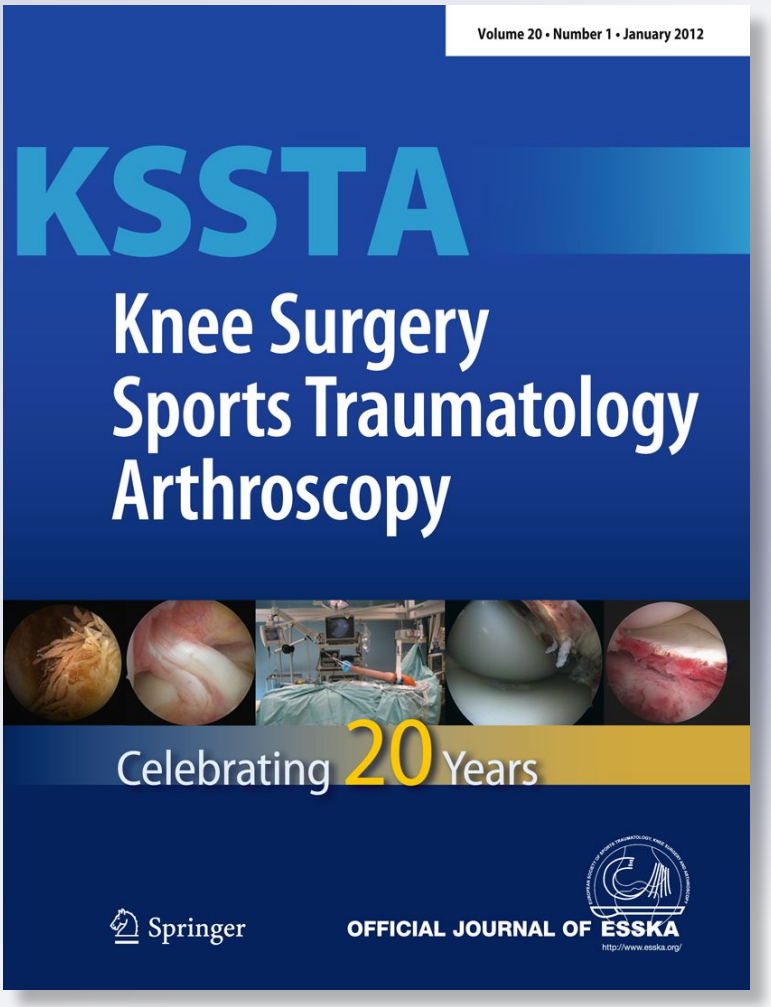
Knee Surgery, Sports Traumatology, Arthroscopy

ISSN 0942-2056

Knee Surg Sports Traumatol Arthrosc
DOI 10.1007/s00167-011-1833-1



Springer

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Effects of graft pretensioning in anterior cruciate ligament reconstruction

Claude Guillard · Francois Lintz · Guillaume Anthony Odri · Denis Vogeli · Fabrice Colin · Sylvie Collon · Daniel Chappard · François Gouin · Henri Robert

Received: 4 May 2011 / Accepted: 13 December 2011

Abstract
Purpose Graft pretensioning is used in anterior cruciate ligament (ACL) reconstruction to prevent secondary slackening. Its effects on collagen fibrillar ultrastructure are not known. In this study, we hypothesized that graft pretensioning in ACL reconstruction creates ultrastructural changes detectable in scanning electron microscopy (SEM).
Methods A prospective comparative study was carried out on 38 ACL reconstructions using a 4-strand semitendinosus graft. Samples were harvested intra-operatively before and after pretensioning for 30 s, 2 or 5 min. The images produced in SEM were analyzed using an original semi-quantitative «CIP» score taking into account collagen cohesion, integrity, and parallelism. Intra- and inter-tester reliability for the CIP score were tested.
Results The CIP scores decreased by 3.5 (1.6) points after pretensioning ($P < 0.05$). Significant differences were found in the 5, 2 min and 30 s subgroups for the global CIP score. Relative decrease (Delta CIP) was significantly higher in the 2 and 5 min subgroups after pretensioning in comparison with the 30 s subgroups. Intra- and inter-tester reliability for the CIP score were 0.85 and 0.92 ($P < 0.05$).
Conclusion Pretensioning ACL grafts resulted in alteration of the collagen fibrillar ultrastructure, detectable using SEM. These results confirm the existence of collagen ultrastructural changes after pretensioning that may be related to its duration.
Level of evidence Prospective comparative study, Level II.

Keywords Pretensioning · Preconditioning · Hamstring tendons · Anterior cruciate ligament · Scanning electron microscopy · Knee

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Introduction

Clinical results of anterior cruciate ligament (ACL) reconstructions reported in the literature are good in more than 80% of patients [4, 14, 27, 28, 38]. However, authors regularly report difficulties in obtaining a hard anterior drawer endpoint or physiological objective laxity measurements in 5–20% of patients [4, 14, 27, 28, 38]. Secondary slackening or loosening of the transplant has been reported to be a possible explanation for this phenomenon [2, 3, 6, 11, 18, 21, 33, 41]. In daily activities, transplants are subject to traction forces up to 150 N during walking and 450 N during running [24, 25, 40]. It has been advocated that this repeated constraining mechanism could be the cause for secondary slackening. Among the solutions introduced to solve this, intra-operative pretensioning has been adopted by many authors [7, 11, 22, 26, 37, 41, 43] to prevent secondary slackening [3, 6, 11, 18, 20–22, 26, 29, 33, 41, 42] or slip between the different components of the transplant at the interfaces [3, 16, 17, 32]. However, pretensioning remains a controversial procedure [5–7, 18, 19, 23, 29, 36, 38, 43], mainly because the scientific protocols

Published online: 05 January 2012

used in publications concern frozen cadaver samples or animal models rather than patients. Results may therefore not be applicable to intra-operative pretensioning techniques [3, 6, 11, 20, 33, 43]. For some authors, pretensioning may even be deleterious [5, 6, 18, 19, 23, 29, 36, 38, 43] through either plastic failure of the graft [19, 38], or failure of integration and remodeling [5, 6, 29], or vascular impairment.

Scanning electron microscopy (SEM) has been promoted by previous authors as a gold standard and a validated tool to study collagen ultrastructural organizations [12, 30, 35, 44]. However, it had not yet been used to study the effects of pretensioning on transplants in ACL reconstruction. Tendons used as grafts in ACL reconstruction are made of dense conjunctive tissue among which collagen fibrils are tightly packed together, regularly dispatched, and oriented parallel to each other [35, 39]. The semitendinosus (ST) tendon has an elongation at plastic failure of 4–8%, according to different authors, and an elongation at rupture of 10% [9, 31, 39]. Wang et al. [39] reported microscopically identifiable fiber ruptures at 4% elongation, which become macroscopically visible at 10%. Previous studies have otherwise made no reports about the effects of pretensioning on the collagen ultrastructure. For this work, it was hypothesized that pretensioning a ST transplant, in an ACL reconstruction technique, is responsible for an alteration of its collagen ultrastructure, which can be identified and quantified using a specific score in SEM. This alteration in collagen ultrastructure may be dependent on the duration of pretensioning.

Materials and methods

A series of 38 patients was included between January 2010 and January 2011. Inclusion concerned any patient referred to our department for ACL reconstruction following isolated ACL rupture. The study was approved by the ethical committee of the Nord-Mayenne Hospital, and written informed consent was obtained from all patients. Exclusion criteria were the decision not to undergo biopsy, any pre-operative report of surgical history or trauma to the hamstring region, and technical failure during the sample harvesting or the processing protocol.

The methodology was prospective and comparative. Two independent and blinded observers conducted data analysis.

The ACL reconstruction technique used was an all inside technique with a 4-strand ST and a polyethylene terephthalate (PET) strip hybrid transplant (Fig. 1). The ST tendon was harvested using an open stripper. The tendon was folded into a 4-strand graft mounted on a PET strip at each end. Diameter and length of each graft were measured with a millimeter caliper. One centimeter long and 1 mm wide samples for SEM assessment were harvested in the mid portion of the graft. The hybrid transplant was then fixed on a dedicated traction ancillary calibrated at 500 N. Traction was applied through the PET strips for 5 min in 17 patients, 2 min in 13 patients, and 30 s in 8 patients, after which the dimensions of the graft were again measured and a new sample harvested for SEM assessment.

The harvested samples were immediately fixed in ready-to-use plastic vials. The fixative was composed of 4% glutaraldehyde in a cacodylate buffer (pH 7.4). Samples were fixed for 24 h at 4°C in the surgical unit then rinsed, stored in a cacodylate buffer, and sent to the laboratory. The samples were then rinsed, defatted in acetone-xylene, and post-fixed in 1% osmium tetroxide in a 0.1 M cacodylate buffer, dehydrated in a graded series of ethanol, and treated by critical point drying in liquid CO_2 for 1 h. They were carbon coated prior to SEM examination at 3–7 kV (Jeol JSM-6301 F). The grayscale pictures produced by SEM (10,000×) were analyzed twice by two independent and blinded observers according to an original 12 points score entitled «CIP score», which took into account semi-quantitative visual analysis of collagen fibrillar cohesion (4 points), integrity (4 points), and parallelism (4 points) (Fig. 2).

Fig. 1 Hybrid graft composed of four strands of semitendinosus plicated and mounted on two strips of polyethylene terephthalate

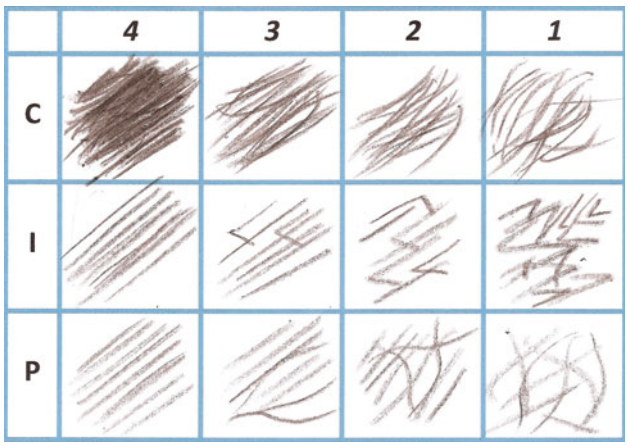

Fig. 2 CIP score (cohesion/integrity/parallelism). Score divided into three items, cohesion, integrity, and parallelism, each scored on four points

Statistical analysis

A normality test was performed (Kolmogorov–Smirnov) on all variables before parametric statistics. Global CIP score, cohesion, integrity, and parallelism were considered as continuous variables. To compare means before and after pretensioning (overall series, 5, 2 min and 30 s), two-way paired *t*-tests assuming equal variances were performed. The variables for the three time groups (5, 2 min and 30 s pretensioning) were compared with two-way unpaired *t*-tests. Pearson's correlation coefficients were calculated between all variables and CIP scores. Inter- and intra-tester reliability were tested by an intraclass correlation coefficient (ICC) for absolute agreement, based on the two-way random effect ANOVA test according to Shrout and Fleiss [34]. A P value less than 0.05 was considered significant. Statistics were done using SPSS 16.0 and Microsoft™ Office Excel 2007 softwares.

Results

Thirty-eight patients were included in the study (30 men and 8 women). Mean age was 25.6 (9.9) years. Mean length of the 4-strand plicated ST graft before pretensioning was 50.7 mm (3.3), and mean diameter was 9 mm (1.2). After pretensioning, mean length was 53.3 mm (3.5). Mean relative stretch was 5.2% (1.6).

The grayscale digital photographs produced typically showed a standard parallel and continuous organization of the collagen fibrils before pretensioning. After pretensioning, disorganization including loss of parallelism, cohesion, and fibril integrity through plication was observed (Fig. 3a, b). The specific directional architecture of collagen fibrils was disrupted in different proportions depending on the time subgroup (Fig. 4a, b). The CIP score decreased by a mean 3.5 points (1.6) after pretensioning ($P < 0.05$) in the global series. A significant ($P < 0.05$) decrease in CIP score was found after pretensioning for 2 and 5 min. This was found for the global CIP score and for each item. In the 30 s pretensioning group, the generic CIP score, the integrity, and parallelism items were significantly altered, but not the cohesion item (Table 1). Comparison of the 30 s, 2, and 5 min groups showed significantly ($P < 0.05$) higher Delta CIP scores (relative decrease of the CIP score after pretensioning) for the 2 and 5 min groups. This was also true for the cohesion item (Table 2). In the 5 and 2 min pretensioning series, respectively 15 and 10 transplants out of 17 and 13 had lost 3 CIP points (>2 Standard Deviations) or more, while only 2 transplants out of 8 had lost 3 CIP points or more in the 30 s pretensioning subgroup.

Age, sex, relative elongation, the diameter of the transplant, and the initial CIP score were not predictive of the final CIP score. The CIP Scores' intra- and inter-tester reliability ICC were respectively, 0.851 [0.689–0.921] ($P < 0.05$) and 0.926 [0.870–0.957] ($P < 0.05$).

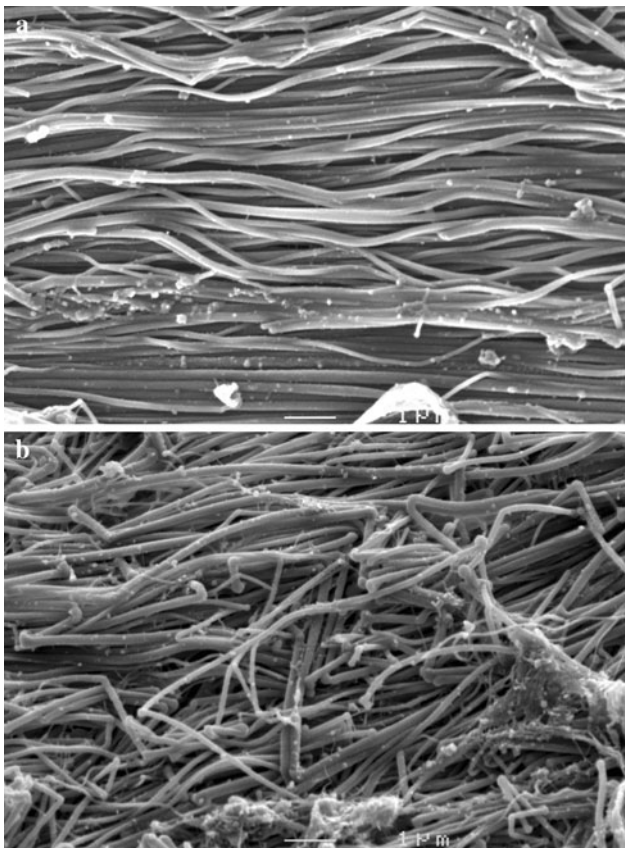

Fig. 3 SEM ×10,000. Graft collagen ultrastructure **a** before pretensioning at 500 N during 5 min. CIP score was 11 points (3/4/4). **b** after pretensioning at 500 N during 5 min. CIP score was 5 points (2/1/2)

Discussion

The most important finding in the present study was that pretensioning a 4-strand hybrid ST and PET transplant in an ACL reconstruction technique at 500 N for more than 30 s created significant alterations of its collagen fibrillar ultrastructure through loss of cohesion, integrity, and parallelism of the collagen fibrils. These modifications were identifiable and quantifiable using SEM and an original score with excellent intra- and inter-tester reliability.

Our findings corroborate observations in the literature [5, 6, 18, 19, 23, 29, 36, 38, 43], which have previously questioned the innocuity of pretensioning procedures in ACL reconstructive surgery.

This is an original, level II, prospective comparative study. The use of SEM, which is a validated technique for ultrastructural analysis of collagen tissues [12, 30, 35, 44],

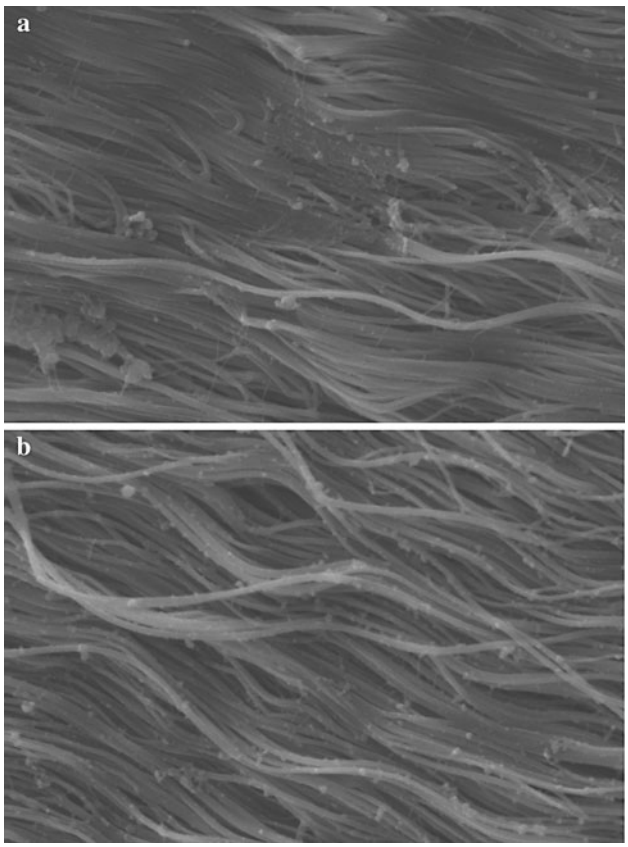

Fig. 4 SEM ×10,000. Graft collagen ultrastructure **a** before pretensioning at 500 N during 30 s. CIP score was 12 points (4/4/4). **b** after pretensioning at 500 N during 30 s. CIP score was 10 points (3/4/3)

has not been reported previously. Also, the use of samples harvested intra-operatively rather than on cadaver or animal tissues was closer to clinical practice. In previous studies, transplant pretensioning in ACL reconstruction was reported to be efficient in post-operative residual anterior laxity by abolishing graft visco-elastic adaptation capabilities. The graft is thus transplanted at its least compliant and most rigid state possible [3, 6, 11, 18, 20–22, 26, 29, 33, 41]. Pretensioning protocols described in the literature include either static axial traction of the transplant on a dedicated ancillary or in situ manual cycling, using the femoral fixation as an anchoring point [22]. These have demonstrated efficiency in producing a more rigid graft and an improvement of post-operative laxity [3, 6, 11, 18, 20–22, 26, 29, 33, 41, 42]. Ancillary-mediated pretensioning also provided more reliable results than manual pretensioning due to better reproducibility [22]. However, these studies measured immediate post-operative laxity, which did not take into account transplant remodeling, integration, and viability.

According to Elias et al. [8], pretensioning at 160 N rather than 80 N creates a stiffer graft and decreases residual laxity. However, it has been advocated that the efficiency of pretensioning in creating a more rigid transplant may only be true below 80 N [1, 26]. Figueroa et al. [10] using porcine tendons have even reported a decrease in resistance in pretensioned transplants. Therefore, some authors advise not to pretension transplants using forces over 40 N to avoid revascularisation impairment [29]. Yoshiya et al. [43] have demonstrated the presence of overconstraining lesions above 30 N, characterized by chondral defects. Other authors found no clinical or functional difference in longer follow-up reports between the pretensioned grafts and the non-pretensioned ones [7, 37, 43], with evidence based on mechanical testing, objective laxity, and functional evaluation. On the whole, a consensus on the kind of pretensioning protocol to use is still to be found and depends on the operative technique used individually [15, 19].

Pretensioning also creates transplant lengthening or stretching. Mean lengthening in our series was 5.2% (1.6), ranging from 1.9 to 8.9%, and was in the elastic zone, which has been defined between 0 and 4% to 0 and 8% by previous authors [9, 31, 39]. This must therefore be put in perspective as far as our results are concerned since we did not measure isolated tendons but the whole hybrid graft containing interfaces (including tendon–tendon, tendon–PET, and tendon–suture interfaces). During pretensioning, the different components of the transplant slip against each other and adjust to equalize the traction forces on each strand and stabilize the tendon–PET interface. Elongation is predominant at the tendon–PET interface [3, 16, 17, 32]. However, a contribution of plastic failure to the total amount of lengthening observed cannot be excluded.

The limitations in this study are the small number of patients and the use of a semi-quantitative score based on optical analysis of gray-scale photographs, which implies a degree of subjectivity. An automatic digital image analysis

Table 1 Results of CIP score in subgroups 2, 5 min and 30 s

	CIP				Cohesion				Integrity				Parallelism			
	Prior	After	Delta	*P*	Prior	After	Delta	*P*	Prior	After	Delta	*P*	Prior	After	Delta	*P*
5 min	10.2	6.5	3.7	<0.01	3.4	2.1	1.3	<0.01	3.2	2.1	1.1	<0.01	3.6	2.3	1.3	<0.01
2 min	10.3	6.3	4.0	<0.01	3.4	1.8	1.6	<0.01	3.3	2.1	1.2	<0.01	3.6	2.5	1.1	<0.01
30 s	9.7	7.5	2.2	<0.05	3.1	2.5	0.6	0.09 (n.s)	3.1	2.4	0.7	<0.05	3.5	2.6	0.9	<0.05

Table 2 Statistical result between the three subgroups

P	ΔCIP	ΔC	ΔI	ΔP
5 vs. 2 min	0.62 (n.s)	0.26 (n.s)	0.84 (n.s)	0.76 (n.s)
5 min vs. 30 s	0.01	0.04	0.18 (n.s)	0.29 (n.s)
2 min vs. 30 s	0.04	0.03	0.15 (n.s)	0.41 (n.s)

system was not applicable at the time of this study in our department, but could be considered for further investigations. Despite this, our results were statistically significant, and the CIP score demonstrated excellent intra- and inter-tester reliability. Any study considering pretensioning may be biased by the use of a specific protocol, which might not be applicable to another surgical team. In fact, the 500 N force used in this protocol is superior to that used in most cases by authors reporting in the literature [1, 7, 21, 26, 29, 37, 43]. Moreover, Hamner et al. measured the ultimate failure at maximum load (UFML) for a one-strand ST tendon at 1,060 N and around 4,000 N for a 4-strand ST tendon graft. Five hundred newtons corresponding to 12.5% of UFML [13] in a 4-strand ST graft and a part of this pretension force is dissipated by lengthening of the PET strip (Stress at rupture: 1,400 ± 150 N and Strain at rupture: 27 ± 5%).

Moreover, observations made on a small sample harvested at the mid portion of the transplant might not account for the whole structure. However, the size of the samples had to be very limited and harvested with great care so as not to compromise the quality of the transplant. Finally, the ultrastructural modifications reported here might not be accountable for altered clinical results, since these observations do not take into account transplant remodeling capacities [6, 29, 44] of grafts in ACL reconstruction.

The observations reported in this work could bring further understanding in the mechanisms of pretensioning through the description and quantification of ultrastructural changes. The degree of disorganization shown here, with loss of fibril cohesion, fibril plication, the loss of orientation and parallelism, may play, to some extent, an important role in the success of graft transplantation. This remains unclear [5, 6, 19, 20, 24, 29, 38, 43].

Conclusion

SEM analysis of intra-operative graft samples in an ACL reconstruction technique using a hybrid ST and PET transplant pretensioned at 500 N for more than 30 s showed ultrastructural disorganization of collagen fibrils. Pretensioning for less than 30 s resulted in less significant alterations. These observations could provide better understanding of the effect of pretensioning on transplants and concur with recent publications, which question the morbidity of pretensioning, concerning graft integration and remodeling capacities. In a clinical setting, based on these observations, the duration of pretensioning should probably not exceed 30 s for an applied force of 500 N.

Acknowledgments The authors wish to thank the staff of SCIAM (Common Service Imaging and Microscopic Analysis, Institute of Biology in Health, Angers University Hospital, Angers 49000, France), R. Filmon, and G. Mabilleau for their support in this research.

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