**Pre-soaking of semitendinosus graft with vancomycin
does not alter its biomechanical properties. A
biomechanical in-vitro controlled study using graft from
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► **To cite this version:**

Christophe Jacquet, Maxime Jaubert, Charles Pioger, Abderahmane Sbihi, Martine Pithioux, et al..
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biomechanical in-vitro controlled study using graft from living donors Running title: Vancomycin Pre-
soaking does not alter Hamstring Biomechanical Properties MD Maxime Jaubert MD. Arthroscopy:
The Journal of Arthroscopy and Related Surgery, 2020, 10.1016/j.arthro.2020.03.037 . hal-02556210

HAL Id: hal-02556210

<https://hal.science/hal-02556210>

Submitted on 27 Apr 2020

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Pre-soaking of semitendinosus graft with vancomycin does not alter its biomechanical properties.

A biomechanical in-vitro controlled study using graft from living donors

Running title: Vancomycin Pre-soaking does not alter Hamstring Biomechanical Properties

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The study has been performed in accordance with the ethical standards in the 1964 Declaration of Helsinki. All patients provided written consent, and the study was approved by our hospital's ethics committee (No. 2016-015724-11; 26 November 2016 and included with the form.

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Pre-soaking of semitendinosus grafts with vancomycin does not alter their biomechanical properties.

A biomechanical in-vitro controlled study using graft from living donors

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Abstract

Purpose:

The aim of this study was to compare the biomechanical properties of human semitendinosus graft pre-soaked with or without vancomycin under a load to failure tensile test.

Methods:

Thirty semitendinosus grafts harvested during ACL reconstruction were included. These were dissected equally into two halves and subsequently randomly allocated to a vancomycin group and to a control group. A digital caliper was used in order to precisely measure each samples thickness, length and width. For the vancomycin group; samples were pre-soaked in a solution of 5mg/ml of vancomycin for a duration of ten minutes and the control group samples were pre-soaked in a physiological serum equally for ten minutes. Mechanical testing was performed on a universal testing machine (Instron 5566-A) after a pre-conditioning of 10 cycles of 1 mm extension and a progression of 10 mm/min to failure for each sample. The stress-strain curve was obtained to determine the elastic modulus (Young's modulus), the Ultimate Tensile Stress (UTS), the Ultimate Tensile Elongation (UTE) before failure and the elasticity limit.

Results:

For the control group, the average Young's modulus value was 4.8 ± 0.8 , the average UTS 25.2 ± 5.2 Mpa, the average percentage of UTE $78 \pm 17\%$ and the average elasticity limit value 17.3 ± 5.3 Mpa. For the vancomycin group, the average young's modulus value was 4.7 ± 0.9 , the average UTS 24.1 ± 6.1 Mpa, the average percentage of UTE $82 \pm 14\%$ and the average elasticity limit value 18.5 ± 5.9 Mpa. No significant difference was observed between the two groups for all investigated parameters.

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Conclusion

Pre-soaking of human semitendinosus graft with vancomycin does not alter its biomechanical properties.

Clinical relevance:

This study demonstrates that vancomycin pre-soaking used in order to prevent post ACL reconstruction septic arthritis does not affect immediate biomechanical properties of semitendinosus tendons.

Key Terms: vancomycin, hamstring, ACL reconstruction, mechanical properties, tensile test, septic arthritis, pre-soaking

INTRODUCTION

Septic arthritis following an anterior cruciate ligament (ACL) reconstruction is a serious complication, with the incidence rate ranging from 0.14 to 3.8%¹⁻⁴. The treatment of this complication required in 33.3% of the cases a graft removal with adjuvant antibiotic (on average 35.4 days) with disastrous functional consequences for the patient⁵. Staphylococci are the most important causative agents in up to 90 % of cases with approximately half of those being identified as coagulase-negative staphylococci (CNS)^{3,6-8}. Some authors relate this high rate of infections by CNS to skin microbial flora contamination during graft harvesting³. Recent studies advocate the pre-soaking of ligament autografts with vancomycin to prevent postoperative septic arthritis. These series discovered a significant reduction in the risk of infection in the group relying on this protocol (ten minutes of pre-soaking with 5mg/ml of vancomycin)^{9,10}. Vancomycin seems to be an ideal choice for prophylactic antibiotics when used topically. It demonstrates a low rate of bacterial resistance on gram positive bacteria¹¹ and has low toxicity on local tissues^{12,13}. Only one animal study in the literature has evaluated the impact of pre-soaking tendons with vancomycin and the possible consequences on their mechanical properties¹⁴. The immediate impact on the biomechanical properties by pre-soaking human ligament autografts with vancomycin has not been studied thus far. The aim of this present study was to compare the biomechanical properties of human semitendinosus graft pre-soaked with or without vancomycin under a load to failure tensile test. The hypothesis was that pre-soaking semitendinosus grafts with vancomycin did not alter their elastic modulus (Young's modulus), Ultimate Tensile Stress (UTS), Ultimate Tensile Elongation (UTE) before failure and elasticity limit.

114

115 **MATERIAL AND METHODS**

116 Biomechanical testing was carried out with 30 semitendinosus tendon grafts harvested
117 during ACL reconstruction surgery with a four stranded hamstring graft (semitendinosus +
118 gracilis) between September 2017 and December 2018. All patients provided written
119 consent, and the study was approved by our hospital's ethics committee.

120 After tunnel drilling and estimating the minimal length of the final graft, samples for testing
121 were obtained by using distal portions of semitendinosus distal part (tibial insertion), if
122 semitendinosus excess was longer than 6 cm. This allowed them to be equally split in two
123 identical samples of at least 3cm which was the minimal length required to conduct
124 mechanical testing.

125 The demographic characteristics (age, gender, height, weight, the sporting level evaluated by
126 the University of California and Los Angeles (UCLA) score of the patients included are
127 summarized in *table 1*.

128 The semitendinosus grafts deemed suitable for testing were stored at -20° for a maximum
129 duration of 15 days before being prepared into samples to go through the mechanical test. It
130 has been shown that this storage protocol does not alter the biomechanical properties of
131 tendons ¹⁵.

132

133 *Preparation of the samples*

134 After 8 hours thawing at ambient temperature (21 °c) ¹⁶ each semitendinosus was prepared
135 into parallelepipeds and then cut in half to obtain two samples of equal length width and
136 thickness. Subsequently each sample was randomly allocated to either a vancomycin group
137 or a saline solution control group (30 samples in each group).

138 To minimize the bias in the preparation of the specimen, a digital caliper (Absolute
139 Digimatic®, MitutoyoTM, Kanagawa, Japan) with a precision of U=0 .001mm was used in
140 order to measure each samples thickness, length and width. These measurements allow
141 section's size calculation for each sample thus permitting stresses and strains estimation

142 during load to failure tests. Each measurement was carried out by two different operators
143 and the average of the two values was recorded.
144 The control group samples were pre-soaked in a physiological serum (NaCl 0.9%) for a
145 duration of ten minutes.
146 Equally the vancomycin group samples were pre-soaked in a solution of 200ml of
147 physiological serum and 1g of vancomycin for duration of ten minutes (5mg/ml
148 concentration). This protocol has already proven it's efficacy in both clinical trials and in vitro
149 studies ^{9,10,17}.

150

151 *Tensile tests (figure 1)*

152 Mechanical testing was performed on a universal testing machine (Instron 5566-A, Instron®,
153 Norwood MA, USA) (Fig. 1). The ends of each specimen were placed in two jaws (1 cm from
154 each end was placed in the two jaws) from previously published method ¹⁸. The mechanical
155 properties were determined within the tissue between the two jaws again using a similarly
156 validated process¹⁹. The length between the two jaws was measured using a digital caliper
157 (Absolute Digimatic®, MitutoyoTM, Kanagawa, Japan). This length (L0) was used to
158 determine the elongation during the test.

159 Specimens were pre-conditioned with 10 cycles of 1 mm extension. The interface of each
160 specimen/jaw was marked with China ink to monitor potential sliding. All specimens were
161 subjected to a tensile test with a progression of 10 mm/min to failure ²⁰.

162 The elongation and the tensile force applied was measured using an acquisition software
163 Bluehill 3 (Instron®, Norwood, MA, USA). The stress-strain curve was then calculated.

164 The following parameters were analysed to compare the samples:

- 165 - Young's modulus (Mpa)
- 166 - The Ultimate Tensile Stress before failure (Mpa)
- 167 - The Ultimate Tensile Elongation (UTE) before failure defined as the length variation
168 from the initial length (L0) to failure, it was expressed as a percentage.
- 169 - The Elasticity Limit (Mpa) defined by the stress from which a sample stops deforming

elastically and in essence demonstrates irreversible deformity.

- The level of the failure was also collected for each test : jaw-specimen interface or inside the specimen

Statistical analysis

The Kolmogorov-Smirnov's test was used to determine the data's normality distribution.

Parametrical tests were used to compare the normally distributed variable (demographical data's and mechanicals properties) between the groups. Non-parametrical tests were used to compare elongation at failure, since this data was not normally distributed.

Two-tailed tests were carried out using PASW Statistics version 20 (SPSS, IBM Inc., Chicago, Illinois). The significance threshold was set at $P < 0.05$. sample size calculations were based on results of the Ollivier et al study²⁰. These calculations showed that at least 25 samples were needed in each group to show a clinically significant difference of 5 Mpa in UTS value before failure between the groups ($\alpha = 0,05$; $\beta = 0,8$ for a mean UTS value before failure of 13 ± 7 Mpa²⁰ for the control group.). Consequently, we included 30 patients per group, which allowed for 5 specimens to be excluded if necessary.

RESULTS

No specimen slippage occurred and the calculations could be performed on all 60 sample tested. Four failures at the jaw-specimen interface for the control group and three for the vancomycin group ($p=0.6$) were observed. The other failure were observed inside the specimen.

The average Young's modulus value for the control group was 4.8 ± 0.8 and for the vancomycin group 4.5 ± 0.9 .

The average Ultimate Tensile Stress value before failure for the group control was 21.2 ± 5.2 Mpa and for the vancomycin group 22.3 ± 6.1 Mpa.

The average percentage of Ultimate Tensile Elongation before failure for the control group was $78 \pm 17\%$ and for the vancomycin group $82 \pm 14\%$. No significant difference was observed between the two groups for all three parameters (respectively $p=0.8$, $p=0.7$, $p=0.8$. (*table 2*)

Analysis of the stress-strain curves confirmed that the samples possessed a ductile behaviour demonstrating 2 distinct phases, a phase of linear elastic deformation and a phase of plastic deformation (*figure 2*). The average elasticity limit value for the control group was 18.3 ± 5.3 Mpa and for the vancomycin group 17.5 ± 5.9 Mpa. No significant difference was observed between the two groups ($p=0.4$)

DISCUSSION

The main finding of this study is that pre-soaking human semitendinosus grafts with vancomycin does not alter its biomechanical properties. The hypothesis was accepted as no significant change was observed for the Young's modulus, UTS, UTE and elasticity limit between the vancomycin group and the control group.

Septic arthritis occurring after an ACL reconstruction is a serious complication, with a reported incidence rate of 0.14% to 1.8%¹⁻³. Recent results of Sonnery-cottet et al ⁴ study demonstrated that this rate could reach 3.8% in a specific population of professional athletes. In a recent meta-analysis, ⁵ 86.9% of the population required a further surgical procedure for the treatment of septic arthritis post ACL reconstruction, and strikingly in 33.3% of the cases the graft required removal, furthermore adjuvant antibiotic therapy lasted on average 35.4 days.

Some in vitro studies have shown significant efficacy of vancomycin pre-soaking to eradicate bacteria in cases of septic arthritis post anterior cruciate ligament reconstruction^{14,17}.

In their animal study on staphylococcus epidermidis infected tendons, Schuttler et al ¹⁴ demonstrated that pre-soaking for 20 minutes with high concentration vancomycin solution (5mg/ml) reduced bacterial growth after 7 days of culture from 85.7% to 0%.

In another study, Perez-prieto et al ¹⁷ showed that for samples contaminated after harvesting and manipulation/preparation, no bacteria was detected after 14 days in culture in a solution of vancomycin (5mg/ml).

The results of in vivo studies are also encouraging. In two controlled studies, Phegan et al ¹⁰ and Pérez-prieto et al ⁹ have compared 285 and 810 patients respectively receiving intravenous antibiotic prophylaxis without topical graft pre-soaking to 1300 and 734 patients receiving the same antibiotic prophylaxis with pre-soaking the graft with vancomycin for 10 minutes (5mg/ml). The postoperative septic arthritis rate decreased from 1.4% to 0% and from 1.85% to 0% for the first and second study respectively. Furthermore, Phegan et al, included only hamstrings grafts whilst Pérez-prieto et al included both hamstrings (84%) and patellar tendon (16%).

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The mechanical properties of ligaments and tendons are related to various morphological parameters (age, living donor vs cadaveric, preservation methods etc), and the method used to induce failure. These reasons would explain the important variability in the values obtained in mechanical testing from previously published series^{20–23}. However, the results of the control group of this study are similar to those obtained by Ollivier et al²⁰ who used an identical mechanical test protocol.

Only one animal study in the literature has evaluated the impact of pre-soaking tendons with vancomycin and the possible consequences on their mechanical properties¹⁴. This study of porcine flexor tendons mirrors the finding of this study and shows no alterations were demonstrable in terms of their biomechanical properties after antibiotic soaking.

This study has some strengths that are important to highlight. It is one of the only study in the literature which investigates the impact of vancomycin on the mechanical properties of a human graft. Secondly the design of the study avoids the bias of inter-individual variability by using 2 groups with grafts from the same individual placed into each group. Lastly, donor grafts were used from living subjects, whilst in comparison previous published series report using either animal or cadaveric specimens^{14,23,24}.

Limitations

Some limitations can be attributed to this study. Firstly, semitendinosus was the only graft to be evaluated, therefore these results cannot represent all grafts used in common practice for ACL reconstruction.

Secondly, the tensile test was performed on frozen grafts, but it is important to note that it has been shown that the mechanical properties of tendons are not affected by freezing if less than 3 cycles of freezing – thawing are performed^{25–27}.

~~Thirdly~~Finally, the fixation can influence the results of these tensile tests²⁷. Jaws made from resin or cryoclamps are difficult to use and their use has not been validated^{28,29}. Shi and al.¹⁸ validated the use of serrated jaws for ligament autograft fixation after comparing them to

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281 other type of jaws. It is this type of jaw that that has been used in this study.
282
283 ~~Despite these limitations, this study demonstrates that vancomycin pre-soaking does not~~
284 ~~affect immediate biomechanical properties of semitendinosus tendon~~ Finally, s. t This in-vitro
285 time zero analysis on non-vascularized specimens didn't analyse the remodelling phase of
286 the ligamentization process and therefore these results must be completed by further
287 histological studies to confirm the safe use of vancomycin pre-soaking at mid and long term
288 time points.

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290 **CONCLUSION**

291 Pre-soaking of human semitendinosus graft with vancomycin does not alter its biomechanical
292 properties

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FIGURE LEGENDS

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Figure 1 Tensile testing machine

Figure 2: Examples of stress-strain curves for a vancomycin sample group (A) and a control sample group (B). The red line corresponds to the elastic limit and the green line to the ultimate tensile stress

TABLES

Table 1: Demographic characteristics of patients included

Age (years)	30 ±8 (19-42)
Sex ratio (F/M)	11/19
Weight (kg)	69 ± 9 (55-85)
Height (cm)	172 ± 7 (163-184)
Sporting level (UCLA score)	9 ± 1 (8-9)

	Control Group	Vancomycin group	Absolute value of mean difference	IC-95%	p-value
Young's modulus	4.8 ± 0.8	4.5 ± 0.9	0.3	[-0.5-0.6]	0.8
Ultimate tensile stress (Mpa)	21.2 ± 5.2	22.3 ± 6.1	1.1	[-1.3-1.5]	0.7
Ultimate tensile elongation (%)	78 ± 17	82 ± 14	4	[-6-8]	0.8
Elasticity limit (Mpa)	18.3 ± 5.3	17.5 ± 5.9	0.8	[-1.4-1.3]	0.4

Table 2: Mean difference for the four analysed parameters between the control group and the vancomycin group

Figure 1

Figure 1 Tensile testing machine

Figure 2

Figure 1 Examples of stress-strain curves for a vancomycin sample group and a control sample group. The red line corresponds to the elastic limit and the green line to the ultimate tensile stress