

1 **How preconditioning and pretensioning of grafts used in Anterior Cruciate**
2 **Ligaments surgical reconstruction are influenced by their mechanical time-**
3 **dependent characteristics: Can we optimize their initial loading state?**

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by

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30 **Word Count:** 2575

31 **Structured Abstract**

32 *Purpose:* Consensus about a pre-implant preparation protocol adaptable to any graft used in
33 Anterior Cruciate Ligament (ACL) reconstruction is still lacking. In fact, there is not agreement on
34 reliable metrics that consider both specific graft dimensional characteristics, such as its diameter,
35 and the inherent properties of its constitutive material, i.e. ligaments or tendons. Aim of the present
36 study was to investigate and propose *the applied engineering stress* as a possible metrics.

37 *Methods:* Preconditioning and pretensioning protocol involved groups of grafts with different
38 section (10 or 32 mm²) and materials (i.e. human patellar and hamstring tendons, and synthetic
39 grafts). A 140 N load was applied to the grafts and maintained for 100 s. Initial stress and following
40 stress-relaxation (a mechanical characteristic that can be related to knee laxity) were specifically
41 analysed.

42 *Findings:* Initial stress, ranging between 4 and 12 MPa, was affected primarily by the graft cross-
43 section area and secondarily by the choice of the graft material. In terms of loss of the initial stress,
44 stress-relaxation behaviour varied instead on a narrower range, namely 13-17%.

45 *Interpretation:* Engineering stress can be identified as the correct metrics to optimize the initial state
46 of each graft to avoid excessive stiffness, laxity or fatigue rupture phenomena.

47

48 **Keywords:**

49 Anterior cruciate ligament; graft; preconditioning; pretensioning; stress relaxation

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54 **Introduction**

55 Anterior Cruciate Ligament (ACL) is one of the major ligaments within the human knee; It plays a
56 critical role in joint stability since it avoids lateral dislocations of the femur with respect to tibia.
57 Injuries of ACL are very common due to its prominent role and alteration of knee biomechanics as
58 well as early development of osteoarthritis (OA) are some of the consequences of ACL damage
59 [40]. These considerations lead clinicians toward ACL surgery reconstructions, in the last decade
60 [32] that however— despite reporting promising short- to mid-term functional performance [39] –
61 are not fully able to prevent premature knee OA [15] as well as to a return of complete physical
62 activities [34].

63 The paradigm at the basis of ACL reconstruction implies the use of grafts to mimic the functional
64 behaviour of the native ligament. The most common approaches primarily rely on autologous grafts
65 - including patellar tendon and hamstring tendon -, biological allografts, xenografts and - mostly in
66 augmentation solutions - bioengineered synthetic grafts [12][44] . However, besides the choice and
67 execution of the surgical technique, there are many aspects that may influence the success of ACL
68 reconstruction, among them: the typology of graft selection and the preparation of the implant.
69 Inappropriate graft properties remain indeed a main issue that limits the long-term success of ACL
70 reconstructions [17]. This aspect is well-known in clinical practice, where excessive laxity can be
71 found in reconstructed knees as the effect of the time-adaptation of the used grafts [9][10]. In
72 particular, the impact of viscoelastic characteristics on graft tensioning is critical [31][37]. In this
73 perspective, preconditioning and pretensioning of the graft aim precisely to eliminate tension and
74 stiffness decay after final fixation, but a shared and validated preconditioning/pretensioning
75 protocol is still lacking [28]. Some recent clinical protocols have been proposed by Zheng et al.
76 [43], that, however is difficult to generalize to different graft diameter from those used by the
77 authors. Indeed, the effect of the initial stress on the hereditariness of different tendons of the

78 human knee is still missing in scientific literature and only limited information about mammalian
79 tendons may be found [31, 40]. Recently the effects of the initial elongations on the relaxation of
80 some kind of fibrous tissue has been studied in stochastic context to assess a numerical predictive
81 model for the mechanical features of the fibrous tissues [3].

82 In order to gain a robust and shared consensus about time-dependent behaviour of grafts used in
83 surgical reconstructions, as first, the correct definitions should be introduced and shared. In this
84 case, despite *tension/stress* appears as the fundamental terms – and in fact we talk about
85 *pretensioning* [43] and *stress relaxation* [28] –, current *preconditioning* protocols are carried out in
86 terms of applied *force*, while *stress* depends on the graft section area, i.e. graft geometrical
87 characteristics.

88 The first assumption of this study is that – focusing on *stress* – a preconditioning protocol can be
89 optimized with respect to the graft section, i.e. diameter, since geometrical factors affect,
90 significantly, the results of the protocol [3]. The second assumption is that the protocol tuning on
91 the graft diameter may be not sufficient to obtain an optimal outcome, because the grafts involves
92 different tissue with different mechanical behaviour, although of the same size.

93 In this experimental study these assumptions are investigated to provide the effects of an “in vitro”
94 mechanical test, mimicking a simple preconditioning/pretensioning protocol, and apply it to several
95 graft samples used in ACL reconstruction. Final aim of this work is to identify reliable metrics able
96 to support the optimal tuning of the initial loading conditions for any graft chosen in the treatment
97 of ACL injury.

98 **Materials and methods**

99 *Specimen selection and preparation*

100 The experimental campaign involved two different types of human tissues, namely patellar (P) and
101 hamstring (H) tendons, and a commercial synthetic graft (S). This choice was justified by the fact

102 that these tissues are commonly used in ACL reconstruction [18]. They were collected from a
103 Tissue Bank (*Science Care*, USA). The study was approved by the local Ethics Committee
104 (protocol “TISS-KNEE” 8425). Tendons were stored at -80 °C and thawed in a 37 °C water bath for
105 15 min prior to testing [16] and then prepared by an experienced orthopaedic surgeon. After
106 preparation, each specimen was cleaned and cut at the same length before clamping (Fig. 1). Four
107 different groups were prepared:

- 108 - “H 32 mm²”, an H group with section 32±11 mm², with pretensioning load of 140±18 N
109 that corresponds to 4 MPa stress, equivalent to the pretensioning load of 160 N used in
110 [43] for 7 mm diameter grafts and indicated as good for stiffness and strength.
- 111 - “H 10 mm²”, an H group with section 10±11 mm², with pretensioning load of 140 N was
112 applied.
- 113 - “P 32 mm²”, a P group with section 32±1 mm², with a pretensioning load of 140 N was
114 applied.
- 115 - “S 10 mm²”, an S group as furnished by the manufacturer with section 10±1 mm², with
116 pretensioning load of 140 N was applied.

117 Each group was composed of six samples, as indicated in [26].

118 *Testing Procedure*

119 The aim of this study is the definition of an “optimal” procedure to tune the initial loading
120 conditions of grafts. The experimental set-up used to this aim has involved the use of a single-axis
121 electroforce dynamic system (Bose 3330, TA instruments) to perform uniaxial tensile tests. Sample
122 principal fibres were qualitatively aligned along the machine loading axis (Fig. 1.d). Clamping was
123 obtained with the aid of milled grips, involving sandpaper for the synthetic grafts and a surgical
124 basting suture at the end of hamstrings grafts (Fig. 1.a) [35]. In order to obtain reliable results, a

125 specific experimental protocol consisting in four phases consecutively applied on the same sample
126 was developed (Fig. 2):

127 1. *Preconditioning*: specimens were preconditioned by harmonic load between 20 and 100 N,
128 for twenty cycles at 0.25 Hz, thus to remove any crimping in the tendon fibrils caused by
129 prolonged storage in a fixed position [23].

130 2. *Recovery*: a 20 N load was maintained for 15 min to exhaust sample elongation. After
131 recovery, the initial length of the specimen as well as its initial width and thickness were
132 measured along three sections of mid-substance by using a standard digital caliper;
133 measurements were repeated three times by two different operators and values were then
134 averaged to calculate strain and engineering stress [25].

135 3. *Pretensioning*: a load was applied to induce pretension on the graft, considering a linear
136 ramp with a loading rate of 315 N/s [13].

137 4. *Stress relaxation*: sample position reached in pretensioning was maintained for 100 s to
138 mimic the behaviour of the grafts being implanted into the knee joint.

139 During the whole experimental test, specimens were continuously moistened with saline
140 solution [22]. Stress relaxation lasted 100 s, that is an observation time chosen for accurate
141 estimation of the phenomenon through a power-law model [33].

142 *Data and Statistical Analysis*

143 Mechanical characterization was obtained in terms of the engineering stress $\sigma(t)$, obtained from the
144 experimental data as $\sigma(t) = \frac{F(t)}{A_0}$, where $F(t)$ is the force measured during the uniaxial test and A_0
145 is the average cross-sectional area measured at the end of the recovery phase.

146 We denoted $\sigma(\underline{t})$ as the stress at the end of loading ramp ($t = \underline{t}$) and therefore modelled the stress
147 relaxation as:

148
$$\frac{\sigma(t)}{\sigma(\underline{t})} = A t^{-\beta} \quad (1)$$

149 where $0 \leq \beta \leq 1$ is a decaying rate, expressing the speed of relaxation, whereas A is a coefficient of
150 proportionality.

151 A specific comparison was carried out between the “H 32 mm²” and “H 10 mm²” groups to
152 highlight the influence of graft diameter. Further, two more comparisons interested the “H 32 mm²”
153 and “P 32 mm²” groups, and the “H 10 mm²” and “S 10 mm²”, respectively, to highlight the
154 influence of graft materials.

155 Comparisons were realized in terms of pretension $\sigma(\underline{t})$, relaxation rate, β , and loading loss at 100 s,
156 $\frac{\sigma(100) - \sigma(\underline{t})}{\sigma(100)}$, and were performed by using *Wilcoxon-Ranksum test* with a level of statistical
157 significance $p\text{-value} = 0.05$.

158

159 **Results**

160 The experimental data from the mechanical tests reporting the normalized stress with superimposed
161 power-law fitting, are reported in Fig. 3.

162 The results about the pretension level $\sigma(\underline{t})$ are collected in Fig. 4. The observation of the data shows
163 that with a three times larger section A_0 , the “H 32 mm²” the group presented about a three times
164 lower $\sigma(\underline{t})$ respect to the “H 10 mm²” set; difference was statistically significant ($p\text{-value} 0.0248$).
165 No difference was present between different materials with same section.

166 The relaxation rates β are collected in Fig.5. It can be observed that samples with larger section, in
167 particular the “H 32 mm²” set, shows faster relaxation if no statistical difference is present.

168 Loading loss, $\frac{\sigma(100) - \sigma(t)}{\sigma(100)}$ (Fig. 6), is strictly connected with β , as expected, since the faster is
169 the relaxation, the higher is the tension decay in a given time interval. Thus, the “H 32 mm²”
170 showed the highest decay, but no statistical difference is present.

171 **Discussion**

172 The first assumption about the influence of the *stress* in the role of the ACL graft cross-section area
173 (i.e. diameter), was specifically demonstrated by comparing grafts of the same typology, i.e.
174 hamstring tendons, including different section areas.

175 The second assumption, about the importance of the graft tissue, arise from the need to tune the
176 preconditioning/pretensioning protocol for each graft. However, the protocol did not show any
177 significantly different effect depending on the graft typology at least for the selected test conditions
178 and analysis.

179 The proposed preconditioning and based-on-*stress* pretensioning protocol shows comparable values
180 of graft loss of tension after fixation, with respect to literature [36][14]. These findings further
181 underlines the strong effect of pretensioning and hence the need to define a proper protocol in
182 clinical applications.

183 The influence of the graft cross-section has been observed primarily on pretension and also, with
184 less extend on stress relaxation. In particular for H, the test yields an inverse relationship between
185 graft area and pretensioning and a direct relationship between graft size and relaxation. The results
186 obtained for P are in agreement with literature [1].

187 The pretensioning load influences the mechanics of the grafts to a large extend [43]. The *Stress*,
188 defined as force on section area, appears as the correct metric to take into account both the
189 contributes, with the aim to identify an optimal approach for graft selection and preparation. In fact,
190 the inherent definition of stress could help comparing and exploiting the results obtained in different

191 studies. For instance, indications about a “dangerous” pretension – i.e. an excessive applied force
192 able to introduce alterations in structure –, seem to be different if we consider a load of 340 N for a
193 hamstring graft [43] and a load of 500 N for a hamstring-polyethylene hybrid transplant [19].
194 Nevertheless, focusing on cross-section area, we found that the first study used a graft diameter of
195 about 7 mm, whereas the second of about 9 mm, thus both leading to a common indication in terms
196 of *stress* (for both about 8 MPa).

197 Indeed, stress is fundamental in interpreting structural-mechanical relationship [5][7][6][8]. The
198 previously reported stress value of 8 MPa represents an important basis also to interpret our results.
199 While Hingorani et al. [27] and Vena et al. [41] showed that a higher pretension load led to a higher
200 level of relaxation, the behaviour identified in this work appeared to be quite different, presenting
201 lower level of relaxation when higher stress was applied; anyhow in [27] only pre-damage strains
202 were used and it was speculated that a separate behaviour may be identified at higher levels of
203 induced stress. This could be indeed our case, since the higher stress values are all above the critical
204 value of 8 MPa, thus micro-damages could have impaired a full reorganization of the tissues and
205 therefore the overall relaxation behaviour.

206 In the analysed testing conditions, the influence of different graft materials was not that evident.
207 Despite few differences due to the proposed testing protocols, consistency with literature was
208 specifically identified in tension level and rate of relaxation for both P and H samples [43][29][1]
209 [24][13]. Unfortunately, no data for comparison were found in literature concerning synthetic graft,
210 with particular attention to the specific polyethylene terephthalate material.

211 Despite this study did not find different macroscopic behaviours related to the several grafts tested,
212 their specificity should not be ignored. Micro-structural differences were in fact reported between
213 hamstring and patellar tendons [20][21]. Although these grafts are of the same typology of tissue,
214 they were harvested from different knee anatomical locations, definitely with a specific different
215 function. For this reason, under mechanical test conditions different from these observed in this

216 study, some significant differences were highlighted [42]. For the analysed synthetic solution,
217 differences with respect to hamstring were not attended in general, as hamstring graft represents a
218 “gold standard”, thanks to a broad range of available analyses [13][30][11][19]. Nevertheless, new
219 materials are under development to obtain optimal ACL graft properties [2], therefore focusing on
220 *stress* - here proposed as one possible tuning metrics - can be useful to identify the optimal graft
221 diameter.

222 This study presented several limitations and that may be improved in the close future. Concerning
223 testing, despite the use of several solutions to avoid slippage of the soft tissues, further experiments
224 should necessarily consider the clamping performed on bone insertions or the use of special
225 gripping techniques [38][4]. The obtained results show that the major limitation pertains to the
226 small sample size and testing conditions, that did not allow us to completely generalize
227 relationships between graft diameters and materials. Nevertheless, this study provided an important
228 basis that can be used as a preliminary approach to optimize in particular the
229 preconditioning/pretensioning protocol and, more in general, the choice of graft in the treatment of
230 ACL injury.

231

232 **Conclusions**

233 A preconditioning and based-on-*stress* pretensioning protocol was proposed to reduce stress decay
234 of ACL grafts after fixation within the knee joint. *Stress*, defined as force on cross-section area,
235 resulted a useful metric to optimize the surgical protocol and can be used to reach a consensus in the
236 preconditioning/pretensioning approach depending on graft choice. Furthermore, the presented
237 findings supported the necessity to analyse the static and time-dependent mechanical behaviour of
238 standard and innovative graft solutions, to increase the basic knowledge about them with the
239 perspective of obtaining optimal clinical and functional outcomes.

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380 **Figure Captions:**

381

382 Figure 1. Preparation for testing of: hamstring tendon sample H (a), patellar tendon samples P (b),
383 synthetic ligament S (c). A P sample clamped in the traction machine (d).

384

385 Figure 2. Scheme of the testing protocol, where F is the vertical force read by the load cell.

386

387 Figure 3. Stress relaxation dimensionless mean curves (markers) for patellar P, hamstring H and
388 synthetic S samples with section area 32 or 10 mm², and relative power fitting superimposed
389 (straight lines). $\sigma(t)$ stands for tension at time t, $\sigma(t_0)$ is the tension at the beginning of the relaxation
390 (pretension).

391

392 Figure 4. Pretension $\sigma(t)$ for patellar P, hamstring H and synthetic S samples with section area 32 or
393 10 mm².

394

395 Figure 5. Relaxation rate β for patellar P, hamstring H and synthetic S samples with section area 32
396 or 10 mm².

397

398 Figure 6. Loading loss, $\frac{\sigma(100)-\sigma(t)}{\sigma(100)}$ for patellar P, hamstring H and synthetic S samples with section
399 area 32 or 10 mm².

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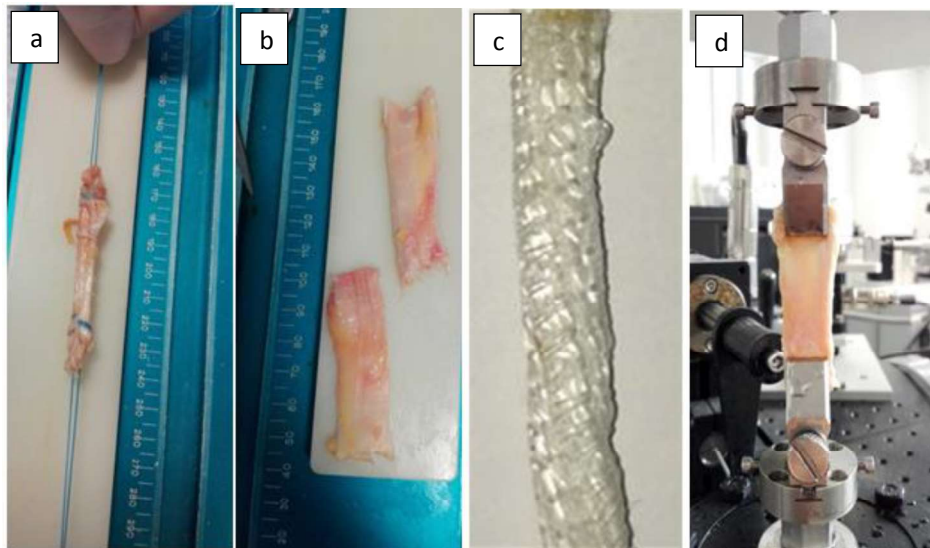
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409 **Figures:**

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411 Fig. 1 a-d

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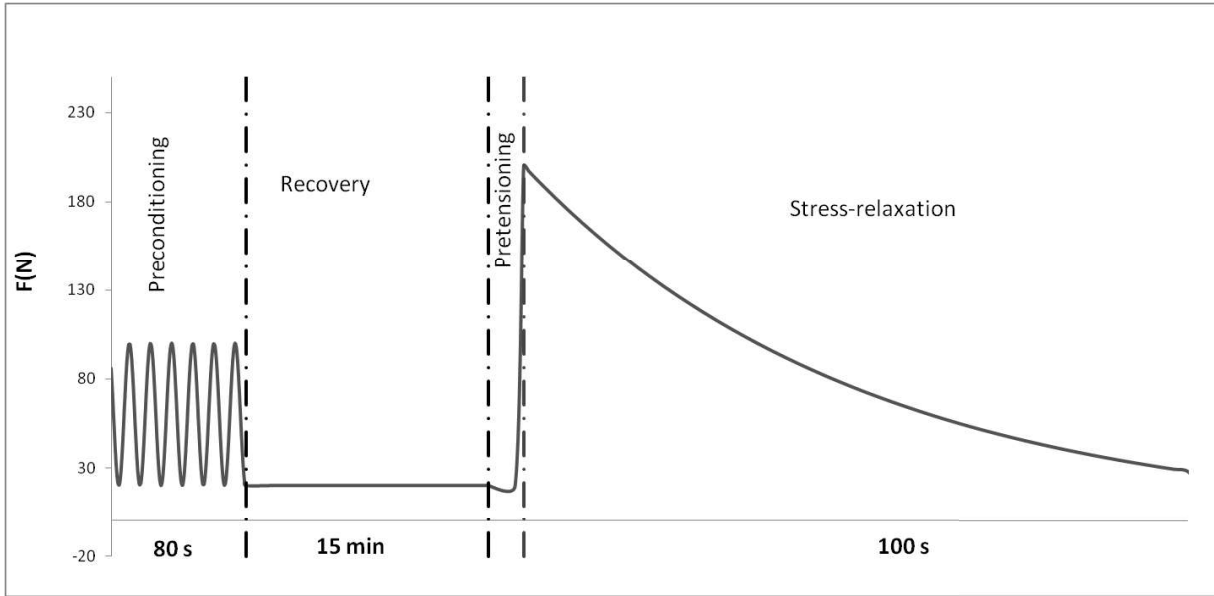


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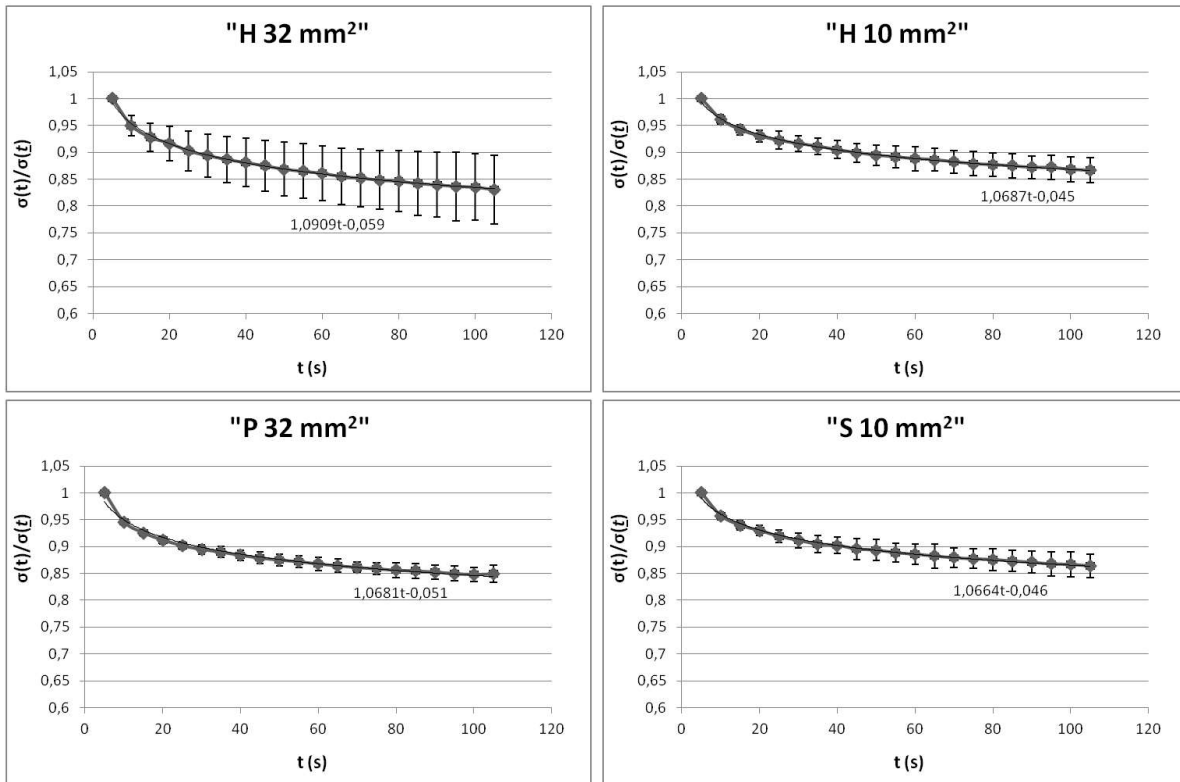
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416 Fig. 2



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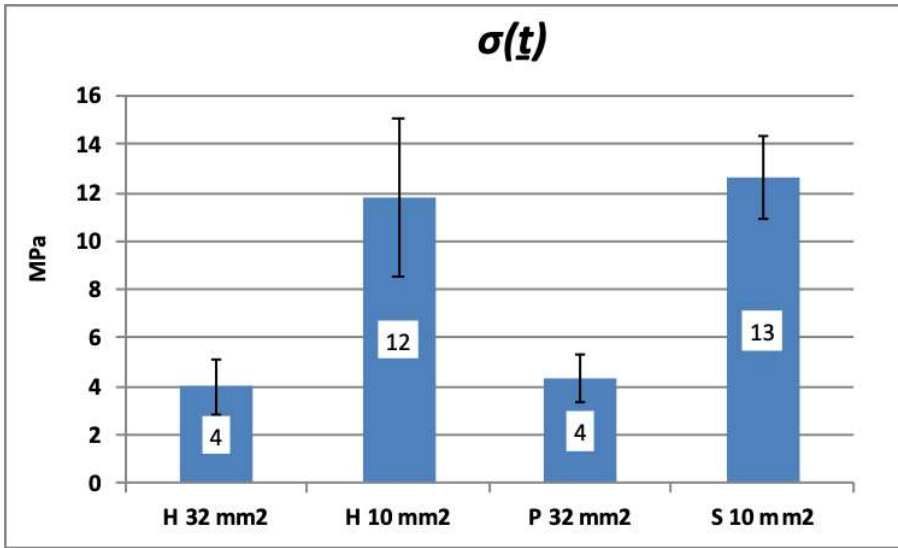
Fig. 3



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429 Fig. 4



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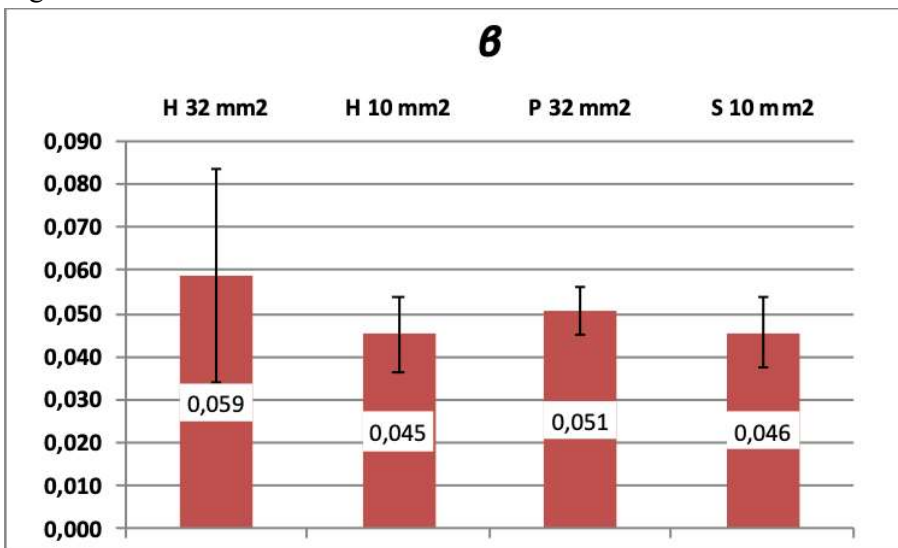
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Fig. 5



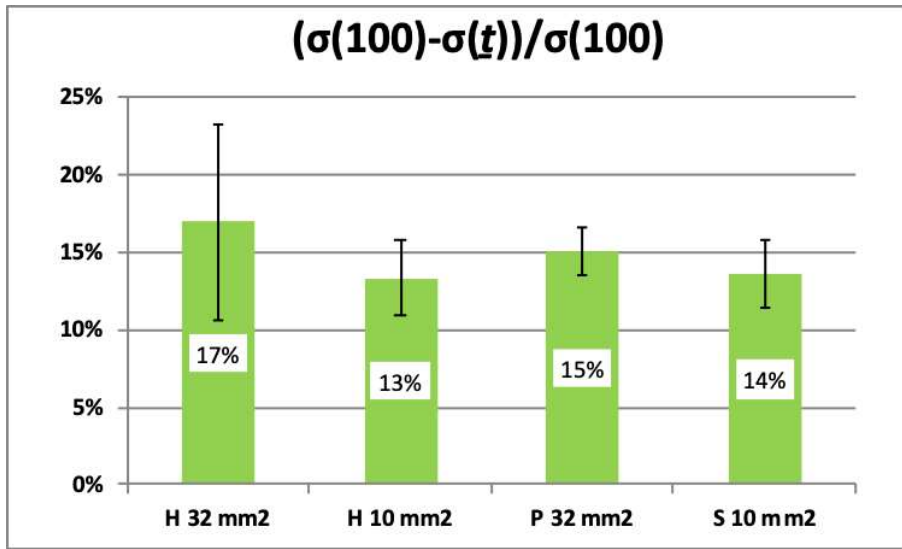
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Fig. 6



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1 **How preconditioning and pretensioning of grafts used in Anterior Cruciate**
2 **Ligaments surgical reconstruction are influenced by their mechanical time-**
3 **dependent characteristics: Can we optimize their initial loading state?**

4

5 by

6

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31 **Structured Abstract**

32 *Purpose:* Consensus about a pre-implant preparation protocol adaptable to any graft used in Anterior
33 Cruciate Ligament (ACL) reconstruction is still lacking. In fact, there is not agreement on reliable
34 metrics that consider both specific graft dimensional characteristics, such as its diameter, and the
35 inherent properties of its constitutive material, i.e. ligaments or tendons. Aim of the present study was
36 to investigate and propose *the applied engineering stress* as a possible metrics.

37 *Methods:* Preconditioning and pretensioning protocol involved groups of grafts with different section
38 (10 or 32 mm²) and materials (i.e. human patellar and hamstring tendons, and synthetic grafts). A 140
39 N load was applied to the grafts and maintained for 100 s. Initial stress and following stress-relaxation
40 (a mechanical characteristic that can be related to knee laxity) were specifically analysed.

41 *Findings:* Initial stress, ranging between 4 and 12 MPa, was affected primarily by the graft cross-
42 section area and secondarily by the choice of the graft material. In terms of loss of the initial stress,
43 stress-relaxation behaviour varied instead on a narrower range, namely 13-17%.

44 *Interpretation:* Engineering stress can be identified as the correct metrics to optimize the initial state
45 of each graft to avoid excessive stiffness, laxity or fatigue rupture phenomena.

46

47 **Keywords:**

48 Anterior cruciate ligament; graft; preconditioning; pretensioning; stress relaxation

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52

53 **Introduction**

54 Anterior Cruciate Ligament (ACL) is one of the major ligaments within the human knee; It plays a
55 critical role in joint stability since it avoids lateral dislocations of the femur with respect to tibia.
56 Injuries of ACL are very common due to its prominent role and alteration of knee biomechanics as
57 well as early development of osteoarthritis (OA) are some of the consequences of ACL damage [40].
58 These considerations lead clinicians toward ACL surgery reconstructions, in the last decade [32] that
59 however– despite reporting promising short- to mid-term functional performance [39] – are not fully
60 able to prevent premature knee OA [15] as well as to a return of complete physical activities [34].

61 The paradigm at the basis of ACL reconstruction implies the use of grafts to mimic the functional
62 behaviour of the native ligament since inappropriate graft properties may limit the long-term success
63 of ACL reconstructions [17]. Indeed this is a critical issue that is usually avoided by preconditioning
64 and pretensioning of the graft to eliminate tension and stiffness decay after final fixation, despite a
65 validated preconditioning/pretensioning protocol is still lacking [28]. Some recent clinical protocols
66 have been proposed by Zheng et al. [43], that, however is difficult to generalize to different graft
67 diameter from those used by the authors. Recently the effects of the initial elongations on the
68 relaxation of some kind of fibrous tissue has been studied in stochastic context to assess a numerical
69 predictive model for the mechanical features of the fibrous tissues [3].

70 In order to gain a robust and shared consensus about time-dependent behaviour of grafts used in
71 surgical reconstructions, as first, the correct definitions should be introduced and shared. In this case,
72 despite *tension/stress* appears as the fundamental terms – and in fact we talk about *pretensioning* [43]
73 and *stress relaxation* [28] –, current *preconditioning* protocols are carried out in terms of applied
74 *force*, while *stress* depends on the graft section area, i.e. graft geometrical characteristics.

75 The first assumption of this study is that – focusing on *stress* – a preconditioning protocol can be
76 optimized with respect to the graft section, i.e. diameter, since geometrical factors affect,

77 significantly, the results of the protocol [3]. The second assumption is that the protocol tuning on
78 the graft diameter may be not sufficient to obtain an optimal outcome, because the grafts involves
79 different tissue with different mechanical behaviour, although of the same size.

80 In this experimental study these assumptions are investigated to provide the effects of an “in vitro”
81 mechanical test, mimicking a simple preconditioning/pretensioning protocol, and apply it to several
82 graft samples used in ACL reconstruction. Final aim of this work is to identify reliable metrics able
83 to support the optimal tuning of the initial loading conditions for any graft chosen in the treatment of
84 ACL injury.

85 **Materials and methods**

86 *Specimen selection and preparation*

87 The experimental campaign involved two different types of human tissues, namely patellar (P) and
88 hamstring (H) tendons, and a commercial synthetic graft (S). This choice was justified by the fact that
89 these tissues are commonly used in ACL reconstruction [18]. They were collected from a Tissue Bank
90 (*Science Care*, USA). The study was approved by the local Ethics Committee (protocol “TISS-
91 KNEE” 8425). Tendons were stored at -80 °C and thawed in a 37 °C water bath for 15 min prior to
92 testing [16] and then prepared by an experienced orthopaedic surgeon. After preparation, each
93 specimen was cleaned and cut at the same length, 2 cm, before clamping (Fig. 1). Four different
94 groups were prepared:

- 95 - “H 32 mm²”, an H group with section 32±11 mm², with pretensioning load of 140±18 N
96 that corresponds to 4 MPa stress, equivalent to the pretensioning load of 160 N used in
97 [43] for 7 mm diameter grafts and indicated as good for stiffness and strength.
- 98 - “H 10 mm²”, an H group with section 10±11 mm², with pretensioning load of 140 N was
99 applied.

100 - “P 32 mm²”, a P group with section 32±1 mm², with a pretensioning load of 140 N was
101 applied.

102 - “S 10 mm²”, an S group as furnished by the manufacturer with section 10±1 mm², with
103 pretensioning load of 140 N was applied.

104 Each group was composed of six samples, as indicated in [26].

105 *Testing Procedure*

106 The aim of this study is the definition of an “optimal” procedure to tune the initial loading conditions
107 of grafts. The experimental set-up used to this aim has involved the use of a single-axis electroforce
108 dynamic system (Bose 3330, TA instruments) to perform uniaxial tensile tests. Sample principal
109 fibres were qualitatively aligned along the machine loading axis (Fig. 1.d). Clamping was obtained
110 with the aid of milled grips, involving sandpaper for the synthetic grafts and a surgical basting suture
111 at the end of hamstrings grafts (Fig. 1.a) [35]. In order to obtain reliable results, a specific
112 experimental protocol consisting in four phases consecutively applied on the same sample was
113 developed (Fig. 2):

114 1. *Preconditioning*: specimens were preconditioned by harmonic load between 20 and 100
115 N, for twenty cycles at 0.25 Hz, thus to remove any crimping in the tendon fibrils caused
116 by prolonged storage in a fixed position [23].

117 2. *Recovery*: a 20 N load was maintained for 15 min to exhaust sample elongation. After
118 recovery, the initial length of the specimen as well as its initial width and thickness were
119 measured along three sections of mid-substance by using a standard digital caliper;
120 measurements were repeated three times by two different operators and values were then
121 averaged to calculate strain and engineering stress [25].

122 3. *Pretensioning*: a load was applied to induce pretension on the graft, considering a linear
123 ramp with a loading rate of 315 N/s [13].

124 4. *Stress relaxation*: sample position reached in pretensioning was maintained for 100 s to
125 mimic the behaviour of the grafts being implanted into the knee joint.

126 During the whole experimental test, specimens were continuously moistened with saline solution
127 [22]. Stress relaxation lasted 100 s, that is an observation time chosen for accurate estimation of
128 the phenomenon through a power-law model [33].

129 *Data and Statistical Analysis*

130 Mechanical characterization was obtained in terms of the engineering stress $\sigma(t)$, obtained from the
131 experimental data as $\sigma(t) = F(t)/A_0$, where $F(t)$ is the force measured during the uniaxial test and
132 A_0 is the average cross-sectional area measured at the end of the recovery phase.

133 We denoted $\sigma(\bar{t})$ as the stress at the end of loading ramp and the stress relaxation is measured in
134 terms of the percentage decay with respect to the initial value with the introduction of the ratio:

$$135 \quad \rho(t) = \frac{\sigma(t)}{\sigma(\bar{t})} = At^{-\beta} \quad (1)$$

136 where $0 \leq \beta \leq 1$ is a decaying rate, expressing the speed of relaxation, whereas A is a non-
137 dimensional coefficient depending on the stress level $\sigma(\bar{t})$ as shown in forthcoming paper.

138 A specific comparison was carried out between the “H 32 mm²” and “H 10 mm²” groups to highlight
139 the influence of graft diameter with the relaxation protocol reported in this study. Further, two more
140 comparisons interested the “H 32 mm²” and “P 32 mm²” groups, and the “H 10 mm²” and “S 10
141 mm²”, respectively, to highlight the influence of graft materials on the decaying ratio. An additional
142 comparison has been reported for specimens “P 32 mm²” and “H 10 mm²” to show that difference in
143 relaxation may be considered strongly dependent on the specific material used but if different
144 diameters are used then the data are affected by this choice.

145 In order to check the influence at 100 s of the different grafts used other comparisons were obtained
146 in terms of pretension $\sigma(\bar{t})$, relaxation rate, β , and loading loss at 100 s introducing the loading loss

147 ratio $r(100) = \frac{\sigma(\bar{t}) - \sigma(100)}{\sigma(\bar{t})} = 1 - A t^{-100\beta}$, and they have been performed by using *Wilcoxon-*
148 *Ranksum test* with a level of statistical significance $p\text{-value} = 0.05$.

149 **Results**

150 The experimental data from the mechanical tests reporting the normalized stress with superimposed
151 power-law fitting, are reported in Fig. 3.

152 The results about the pretension level $\sigma(t)$ are collected in Fig. 4. The observation of the data shows
153 that with a three times larger section A_0 , the “H 32 mm²” the group presented about a three times
154 lower $\sigma(t)$ respect to the “H 10 mm²” set; difference was statistically insignificant ($p\text{-value} 0.0248$).
155 No difference was present between different materials with same section.

156 The relaxation rates β are collected in Fig.5. It can be observed that samples with larger section, in
157 particular the “H 32 mm²” set, shows faster relaxation as no statistical difference is present.

158 Loading loss, $r(100) = \frac{\sigma(\bar{t}) - \sigma(100)}{\sigma(\bar{t})} = 1 - A t^{-100\beta}$ (Fig. 6), is strictly connected with β , as
159 expected, since the faster is the relaxation, the higher is the tension decay in a given time interval.
160 Thus, the “H 32 mm²” showed the highest decay, but no statistical difference is present.

161 **Discussion**

162 The first assumption about the influence of the *stress* in the role of the ACL graft cross-section area
163 (i.e. diameter), was specifically demonstrated by comparing grafts of the same typology, i.e.
164 hamstring tendons, including different section areas. Indeed, beside the influence of the cross-section
165 area on the value of the stress, a specific influence on the rate of decay on the stress level have been
166 observed.

167 The second assumption, about the importance of the graft tissue, arise from the need to tune the
168 preconditioning/pretensioning protocol for each graft. However, the protocol did not show any

169 significantly different effect depending on the graft typology at least for the selected test conditions
170 and analysis. Indeed, as we compare the results of the starting protocol for similar condition a
171 negligible difference in value of the decay order is observed. Such a comment yields that for clinical
172 application only the value of the initial prestress may be considered when patellar or hamstring
173 tendons are used as driving information for the surgery.

174 The proposed preconditioning and based-on-*stress* pretensioning protocol shows comparable values
175 of graft loss of tension after fixation, with respect to literature [36][14]. These findings further
176 underline the strong effect of pretensioning and hence the need to define a proper protocol in clinical
177 applications. In this regard data provided in this study may be used as a guide for the surgeon to apply
178 a specific value of the pretension force as the cross section of the graft has been measured before the
179 replacement of ACL.

180 The influence of the graft cross-section has been observed primarily on pretension and also, with less
181 extent on stress relaxation. In particular for H, the test yields an inverse relationship between graft
182 area and pretensioning and a direct relationship between graft size and relaxation. The results obtained
183 for P are in agreement with literature [1].

184 The pretensioning load influences the mechanics of the grafts to a large extent [43]. Stress, appears
185 is the correct metric to take into account both the contributes, with the aim to identify an optimal
186 approach for graft selection and preparation. Indeed, the inherent definition of stress could help
187 comparing and exploiting the results obtained in different studies. For instance, indications about a
188 “dangerous” pretension – i.e. an excessive applied force able to introduce alterations in structure –,
189 seem to be different if we consider a load of 340 N for a hamstring graft [43] and a load of 500 N for
190 a hamstring-polyethylene hybrid transplant [19]. Nevertheless, focusing on cross-section area, we
191 found that the first study used a graft diameter of about 7 mm, whereas the second of about 9 mm,
192 thus both leading to a common indication in terms of *stress* (for both about 8 MPa).

193 Such consideration is known in biomechanics since stress is fundamental in interpreting structural-
194 mechanical relationship [5][7][6][8]. The previously reported stress value of 8 MPa represents an
195 important basis also to interpret our results. While Hingorani et al. [27] and Vena et al. [41] showed
196 that a higher pretension load led to a higher level of relaxation, the behaviour identified in this work
197 appeared to be quite different, presenting lower level of relaxation when higher stress was applied;
198 anyhow in [27] only pre-damage strains were used and it was speculated that a separate behaviour
199 may be identified at higher levels of induced stress. This could be indeed our case, since the higher
200 stress values are all above the critical value of 8 MPa, thus micro-damages could have impaired a full
201 reorganization of the tissues and therefore the overall relaxation behaviour.

202 In the analysed testing conditions, the influence of different graft materials was not that evident.
203 Despite few differences due to the proposed testing protocols, consistency with literature was
204 specifically identified in tension level and rate of relaxation for both P and H samples [43][29][1]
205 [24][13]. Indeed unfortunately, no data for comparison were found in literature concerning synthetic
206 graft, with particular attention to the specific polyethylene terephthalate material. The results involving
207 relaxation of P,H and S grafts showed that:

- 208 1) The extent of relaxation rate is strongly influenced by the stress level in the order of decay β .
- 209 2) A quite similar biomechanical behaviour has been observed for the considered tendons P, H
210 and the artificial graft S as prestress increment corresponds to lower load loss ratio at 100 s
- 211 3) The value of the decay's order β is similar for the considered tissues, P,H, S as the same value
212 of pretension is applied to the specimen.

213 Despite this study did not find different macroscopic behaviours related to the several grafts tested,
214 their specificity should not be ignored. Micro-structural differences were in fact reported between
215 hamstring and patellar tendons [20][21]. Although these grafts are of the same typology of tissue,
216 they were harvested from different knee anatomical locations, definitely with a specific different

217 function. For this reason, under mechanical test conditions different from these observed in this study,
218 some significant differences were highlighted [42]. For the analysed synthetic solution, differences
219 with respect to hamstring were not attended in general, as hamstring graft represents a “golden
220 standard”, thanks to a broad range of available analyses [13][30][11][19]. Nevertheless, new materials
221 are under development to obtain optimal ACL graft properties [2], therefore focusing on *stress* - here
222 proposed as one possible tuning metrics - can be useful to identify the optimal graft diameter.

223 This study presented several limitations and that may be improved in the close future. Concerning
224 testing, despite the use of several solutions to avoid slippage of the soft tissues, further experiments
225 should necessarily consider the clamping performed on bone insertions or the use of special gripping
226 techniques [38][4]. The obtained results show that the major limitation pertains to the small sample
227 size and testing conditions, that did not allow us to completely generalize relationships between graft
228 diameters and materials. Nevertheless, this study provided an important basis that can be used as a
229 preliminary approach to optimize in particular the preconditioning/pretensioning protocol and, more
230 in general, the choice of graft in the treatment of ACL injury.

231

232 **Conclusions**

233 A preconditioning and based-on-*stress* pretensioning protocol was proposed to forecast stress
234 decrease of ACL grafts after fixation within the knee joint. *Stress*, defined as force on cross-section
235 area, resulted a useful metric to optimize the surgical protocol and can be used to reach a consensus
236 in the preconditioning/pretensioning approach depending on graft choice. Furthermore, the presented
237 findings supported the necessity to analyse the static and time-dependent mechanical behaviour of
238 standard and innovative graft solutions, to increase the basic knowledge about them with the
239 perspective of obtaining optimal clinical and functional outcomes.

240

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379 **Figure Captions:**

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381 Figure 1. Preparation for testing of: hamstring tendon sample H (a), patellar tendon samples P (b),
382 synthetic ligament S (c). A P sample clamped in the traction machine (d).

383

384 Figure 2. Scheme of the testing protocol, where F is the vertical force read by the load cell.

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386 Figure 3. Stress relaxation dimensionless mean curves (markers) for patellar P, hamstring H and
387 synthetic S samples with section area 32 or 10 mm², and relative power fitting superimposed (straight
388 lines). $\sigma(t)$ stands for tension at time t , $\sigma(\underline{t})$ is the tension at the beginning of the relaxation
389 (pretension).

390

391 Figure 4. Pretension for patellar P, hamstring H and synthetic S samples with cross-sectional area of
392 32 or 10 mm².

393

394 Figure 5. Relaxation rate β for patellar P, hamstring H and synthetic S samples with cross-sectional
395 area of 32 or 10 mm².

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397 Figure 6. Loading loss at 100 s for patellar P, hamstring H and synthetic graft S samples with cross-
398 sectional area of 32 or 10 mm².

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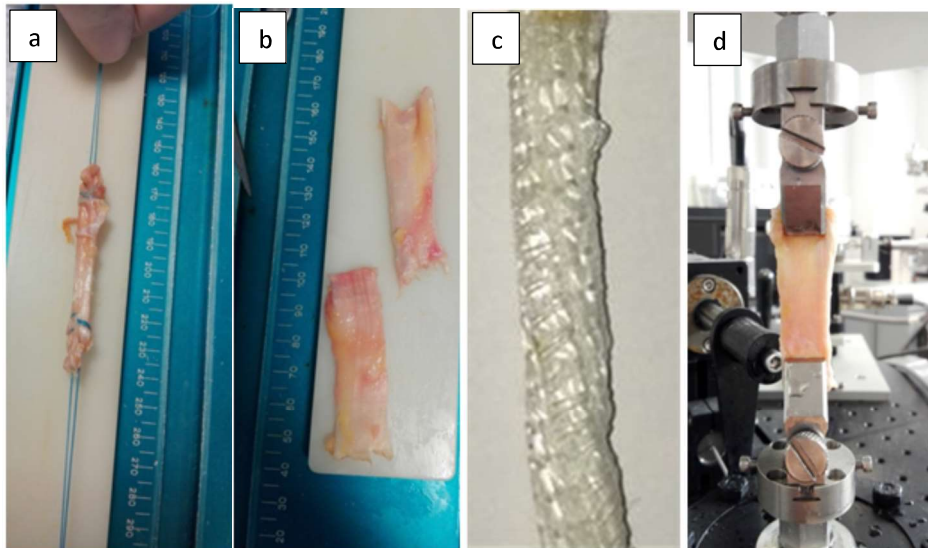
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401 **Figures:**

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403 Fig. 1 a-d

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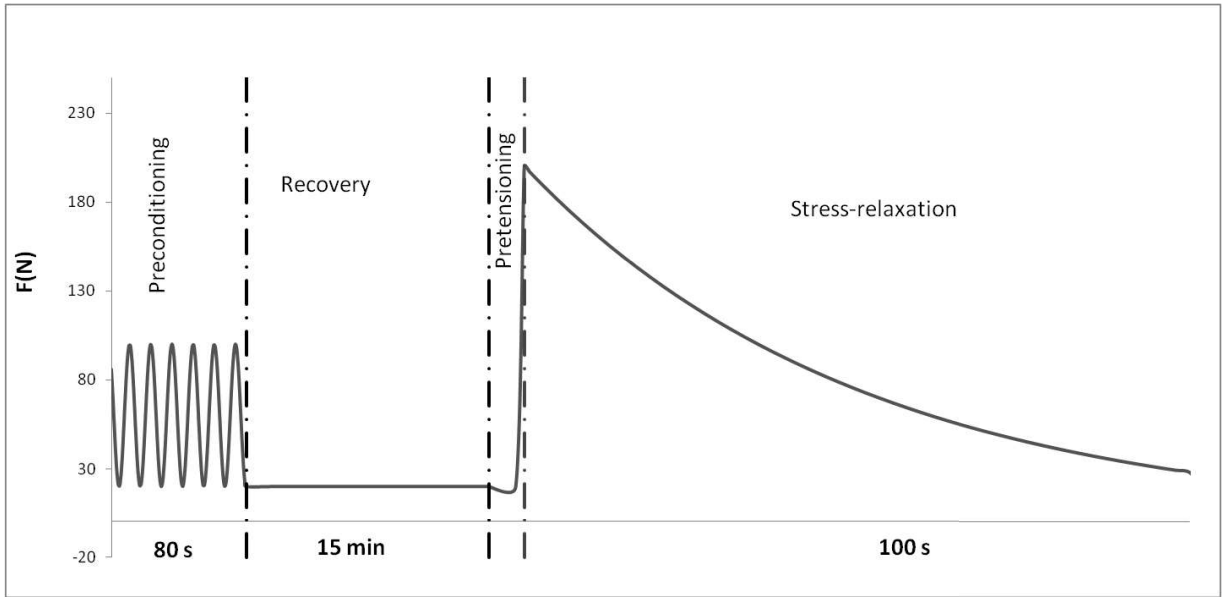


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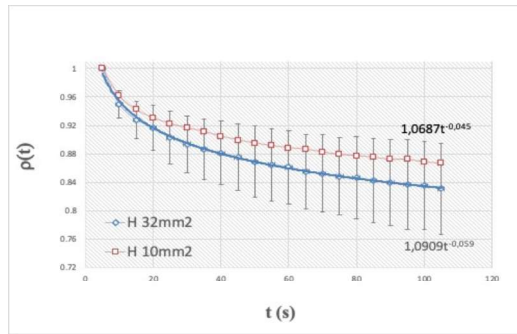
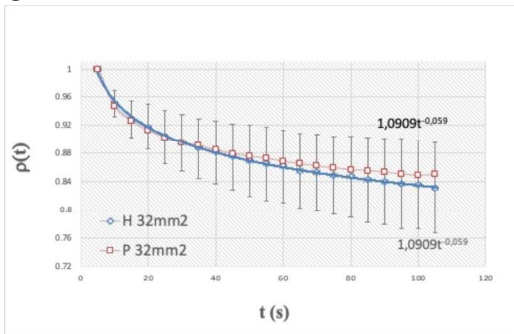
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408 Fig. 2

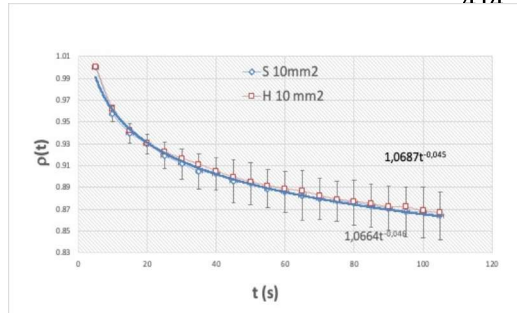
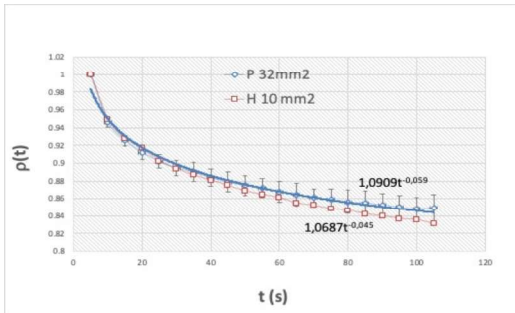


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Fig. 3



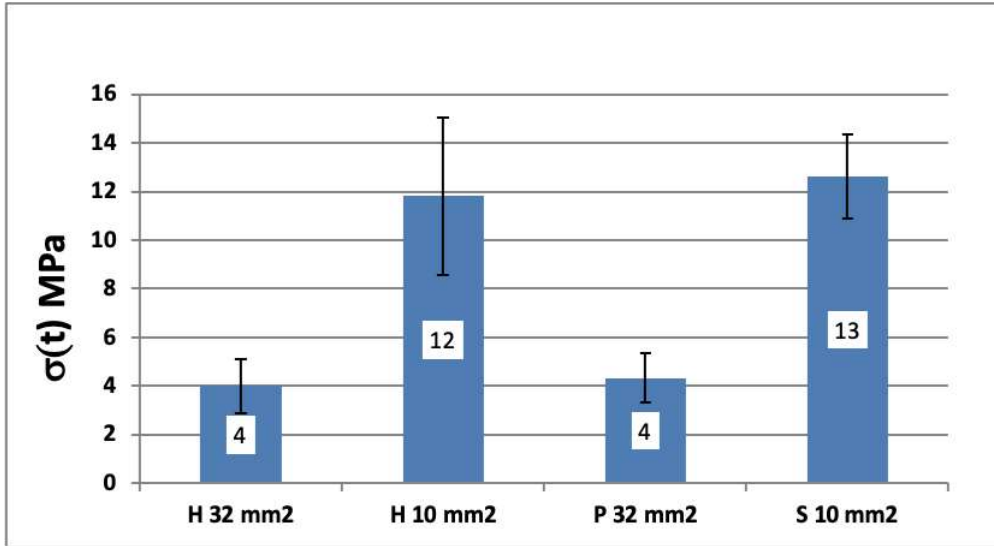
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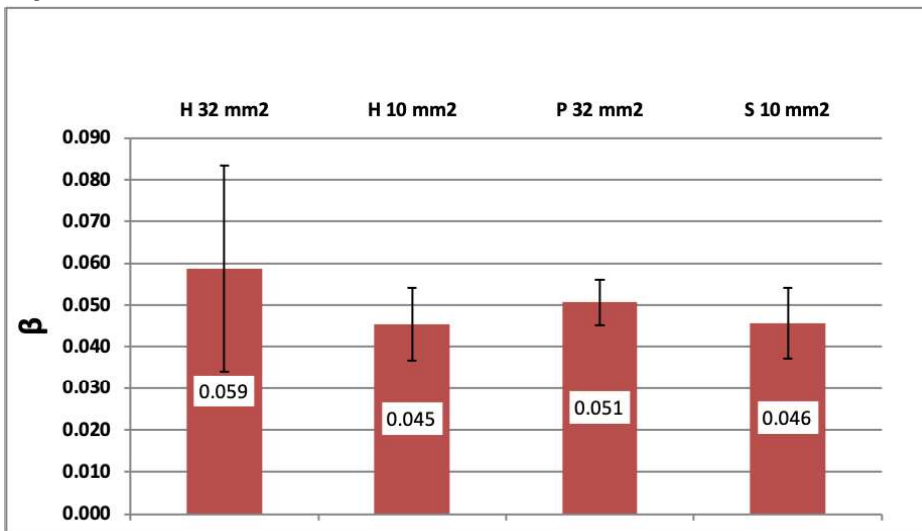
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432 Fig. 4



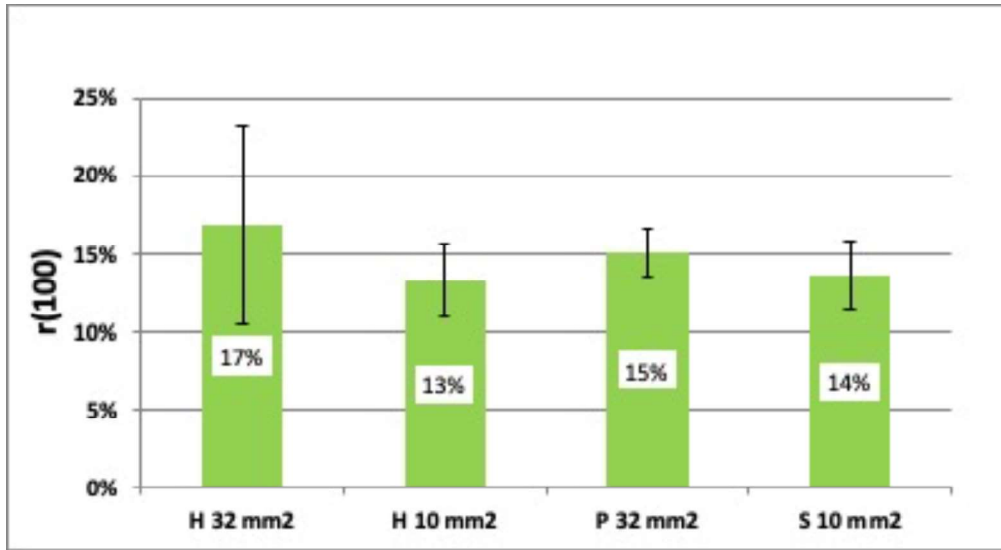
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Fig. 5



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451 Fig. 6



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