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1 **Adapting mechanical characterization of a biodegradable**
2 **polymer to physiological approach of Anterior Cruciate**
3 **Ligament functions**

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15

16 **Abstract**

17 Background and objective

18 Over the past decades, anterior cruciate ligament injuries have become a considerable
19 public health issue. Due to specific physiological conditions such injuries often demand
20 replacement surgery and can take up to two years to a complete recovery. Using
21 biomaterials able to accelerate the healing process could represent a remarkable
22 progress in the field. The main goal of this article is, therein, to evaluate the mechanical
23 properties of poly(ϵ -caprolactone) (PCL) fibers with biological properties enhanced by
24 poly(sodium styrene sulfonate) (PNaSS) grafting when subjected to mechanical stress in
25 different conditions.

26 Materials and methods

27 PCL fibers were thermal grafted with PNaSS. The grafting density was estimated by the
28 toluidine blue colorimetric assay (TB). The influence of the grafting on in vitro primary
29 ACL fibroblast behavior was evaluated by cell proliferation and fluorescence
30 microscope images. The mechanical behavior was evaluated by tensile experiments in
31 air and water, fatigue experiments and simulated walk efforts.

32 Results

33 The results show that poly(ϵ -caprolactone) bundles have their mechanical behavior
34 changed by the different surface treatments and nature of mechanical stress. Although,
35 compared with the values of the natural ligament, the poly(ϵ -caprolactone) has shown
36 superior mechanical properties (Young's Modulus, elastic deformation and ultimate
37 tensile stress) in all studied scenarios. In addition, the pNaSS-grafted surfaces presented
38 a positive influence in the cell proliferation and morphology.

39 Conclusion

40 The pNaSS-grafted PCL has responded mechanical and biological requests for suitable
41 ligament prosthesis material and could be considered as a promising alternative for
42 ACL reconstruction.

43 **Key-words:** Anterior Cruciate Ligament, biodegradable polymer, surface
44 functionalization, mechanical characterization, walking simulation.

45 **1. Introduction**

46 Anterior cruciate ligament (ACL) partial or total ruptures have become more
47 frequent in recent years with the popularization of intense sports activities. In the USA,
48 over 100,000 ACL medical procedures are performed each year, an incident rate of
49 1/3000 [1]. Unfortunately, in the case of a complete rupture, the spontaneous healing
50 process is almost impossible [2]. Given this problem, different techniques and
51 biomaterials have been used to replace the functions of the ACL [2 - 4]. However, the
52 poor choices of materials for the first generation of prosthesis, especially carbon and
53 PTFE based [5], led to problems related to both mechanical behavior and
54 biocompatibility such as ligament rupture and fixation issues, debris amassing, bone
55 tunnel enlargement and inflammation [4, 6, 7] . In response to this failure, synthetic
56 prostheses were developed using polymers, mainly poly(ethylene terephthalate) (PET)
57 [8]. However, PET ligaments still have limitations in biointegration and mechanical
58 properties [9], and most importantly, face a strong resistance from healthcare
59 professionals.

60 At this point, finding a slowly degradable biomaterial that combines good
61 biocompatibility and mechanical properties similar to the natural ligament has become
62 the key point of several studies [10 - 14]. In this context, the tissue engineering field
63 started working towards developing an innovative type of prosthesis serving as a

64 support for a new ligament growth [15, 16]. The objective is to use biodegradable
65 material to guide the generation of a new tissue that gradually replaces it [17].
66 Therefore, to reduce the problems of tissue regeneration due to the constraints realized
67 over the prosthesis, it is necessary that the synthetic ligament gradually loses its rigidity
68 and while the newly formed ligament gains it [8]. Only few materials are able to fulfill
69 all these stipulations and a fortuitous example is the Poly(caprolactone). This semi-
70 crystalline and biodegradable polymer presents total degradation time as high as four
71 years depending on the starting molecular weight [18 - 20] and is a promising candidate
72 for long-term implantable applications, due to its compatible mechanical properties such
73 as Young's Modulus, elastic deformation and rupture strength and well-known
74 biocompatibility [21].

75 Nevertheless, besides the relevance of these bulk properties, the success of a
76 prosthesis implantation in the biological environment is closely linked to surface
77 characteristics and different surface treatments were proposed to increase polymeric
78 prosthesis efficiency, such as glow discharge treatments, plasma assisted modifications
79 or grafting with bioactive molecules, which allows positive interaction with the body
80 [22].

81 Poly(sodium styrene sulfonate) (PNaSS) grafting of implants surfaces has
82 demonstrated a positive influence on the cell response, over several surfaces for both in
83 vitro and in vivo assays [23 - 26]. Significant results were obtained for PET ligaments
84 in terms of cell spreading and cell adhesion strength [27, 28] and the same tendency has
85 been verified for poly(ϵ -caprolactone) PNaSS grafted samples [29].

86 Concerning the mechanical properties of PNaSS grafted poly(ϵ -caprolactone), a
87 previous study has shown that poly(ϵ -caprolactone) fibers present suitable behavior with
88 superiors Young modulus, elastic deformation and ultimate tensile stress (UTS) when

89 compared to native ACL values [29]. However, the mechanical tests carried out during
90 this study didn't consider the nature of the efforts that ACL is subject to daily, making
91 additional tests necessary to complement the characterization.

92 Simulating real situations of the ligament in the knee joint is a way to come
93 closer to physiological conditions and a strongly relevant option to mechanical
94 characterization of artificial ligaments. The present study aims to go deeper into the
95 mechanical characterization of the artificial ligaments material, before and after surface
96 functionalization, by performing tests in different environments and mechanical
97 solicitations modes. Poly(ϵ -caprolactone) fibers were then subjected to tensile tests in an
98 aqueous environment, fatigue tests and a new protocol was established: the walk cycle
99 experiment.

100 **2. Material and methods**

101 **2.1 Samples Preparations**

102 The poly(ϵ -caprolactone) bundles were assembled from 20 poly(ϵ -caprolactone)
103 fibers (MDB Texinov, France). The fibers were 100 μ m diameter and the bundles were
104 delimited by handmade ties. To eliminate the spin finish components the bundles were
105 submitted to ethyl ether Soxhlet extraction (6 h at 30 °C). Spin components free poly(ϵ -
106 caprolactone) bundles were then PNaSS grafted. This operation was carried out in two
107 steps: surface activation in by ozonation (BMT 802 N ACW, oxygen flow rate of 0.6
108 L/min) at 30°C in distilled water for 10 min; and radical polymerization of NaSS from
109 the surface (0.7M H₂O) at 45 °C for 1 h.

110 All samples were washed in distilled water for 48 hours and dried under vacuum before
111 assays.

112 **2.2 Materials characterization**

113 **2.2.1 Toluidine Blue colorimetric assay**

114 The PNaSS grafting density was assessed using the toluidine blue O colorimetric
115 assay. The method was previously described before by Helary et al. [30]. Briefly,
116 samples were soaked in 5 mL of toluidine blue O (TB) 50mM aqueous solution (Roth
117 Sochiel, Germany) and kept at 30°C for complexation reaction. After 6 hours the
118 samples were washed three times in 1mM NaOH aqueous solution for 5 min. Finally,
119 each sample was transferred to a vial containing 2mL of aqueous acetic acid solution
120 (50%, v/v) and kept at room temperature for 24 hours of decomplexation. The
121 absorbance (abs) of the resulting solution was measured by UV/visible spectroscopy
122 (Perkin Elmer Lambda 25 spectrometer, Waltham, USA) at 633 nm and compared to a
123 standard curve of known TB concentration. The grafting density (GD) in mol/g was
124 calculated following Equation 1:

$$125 \quad GD = \frac{abs V}{\epsilon l S}$$

126 The aqueous acetic acid volume is called V (L), ϵ is the angular coefficient of toluidine
127 blue standard curve solution (L/(mol cm)), l is the length of the spectrophotometer path
128 length (cm), and S is the surface area of the samples (cm²).

129 **2.2.2 Differential Scanning Calorimetry**

130 For the thermal characterization, 2 mg of fibers were positioned in an aluminum
131 crucible and placed into a DSC 8000 calorimeter (Perkin Elmer, USA) under nitrogen
132 atmosphere. All scans were performed from -75 to 100 °C, at 10 °C/min. The
133 crystallinity degree was estimated as a ratio of melting enthalpy of the samples (ΔH_m)

134 and melting enthalpy of 100% crystalline poly(ϵ -caprolactone) (ΔH_{m0}) [31] as showed
135 in the equation 2.

$$136 \quad X_c = \left(\frac{\Delta H_m}{\Delta H_{m0}} \right) 100$$

137 **2.2.3 Atomic Force Microscopy**

138 The topography of the polycaprolactone fiber surface was observed by AFM
139 MultiMode 8 (Bruker, Billerica, USA) and analyzed by NanoScope Analysis 1.8
140 (Bruker) using the method ScanAsist in the air ($f= 0.5$ kHz, $0.5 \mu\text{m} \times 0.5 \mu\text{m}$ and $5 \mu\text{m}$
141 $\times 5 \mu\text{m}$ scans). A single tip was used ($k = 0.4$ N/m, tip radius < 10 nm) for the
142 measurements. Peakforce QNM was used to map the adhesion (room conditions) and
143 absolute calibration was used. Gaussian fit was used for calculate the adhesion force.

144 **2.3 Cell culture**

145 Before cell culture the samples were widely washed in salt solution (NaCl 1.5M, NaCl
146 0.15M and PBS), rinsed in ethanol 70% v/v solution for 20 minutes and irradiated with
147 UV under a flow hood for 15 minutes each side. The samples were then conditioned in
148 DMEM for 8 hours and stored in complemented DMEM (10% Bovine fetal serum, 1%
149 streptomycin-penicillin, 1% L-glutamine) overnight at 37 °C. Anterior cruciate
150 ligaments cells (sACL) were isolated from a Merino sheep (2-year old female) in
151 accordance with the German legislation on protection of animals and the NIH
152 Guidelines for the Care and Use of Laboratory Animals (NIH Publication 85-23, Rev.
153 1985). The sampling was, as well, approved by the local governmental animal care
154 committee. For isolate the cells, the tissue were cut into of 1 mm² pieces, washed in
155 PBS three times, and incubated for 6 h in 0.1% w/v collagenase solution at 37°C under
156 5% CO₂. The suspension was centrifuged 3 min at 1,500 rpm, the supernatant was

157 disposed, the cells suspended in complete DMEM and cultured in T-75 flasks until
158 confluence. All experiments were performed with cells under passage 5.

159 **2.3.1 Cell proliferation**

160 Grafted and non-grafted samples were placed in a 24 wells plate and seeded
161 with 1ml of cell suspension (50,000 cells/ml). The plates were incubated at 37°C under
162 5% CO₂ and cultured for 1, 3 and 7 days, changing the culture media every 48 hours.
163 After each point was reached the samples were rinsed twice in PBS, transferred to a new
164 well. The cells were detached by trypsination and counted using a Scepter Cells Counter
165 (Merck, USA).

166 **2.3.2 Cell morphology**

167 The cell morphology over grafted and non-grafted samples was evaluated by
168 fluorescence microscopy after 24 hours of culture. The samples were rinsed in PBS and
169 fixed in 4% formaldehyde solution for 10 minutes at room temperature. The samples
170 were then rinsed in PBS three times and stained with 4',6-diamidino-2-phenylindole
171 (DAPI, 1/500 dilution) and fluorescein isothiocyanate (FTIC, 0.13 mg/ml) for 5 minutes
172 at room temperature. The samples were once again rinsed in PBS three times, allowed
173 to dry and the images were acquired using a LSM 710 confocal laser scanning
174 microscopy (Zeiss, Germany).

175 **2.4 Mechanical characterization**

176 **2.4.1 Tensile experiments**

177 Tensile experiments were realized using a Bose Electroforce 3230 test
178 instrument (Bose, USA) with a 450 N force sensor. Poly(ϵ -caprolactone) bundles
179 submitted to classic traction assays were assembled from 20 fibers (100 μ m diameter).

180 The cross section was calculated by multiplying the cross-section of a single fiber by
181 twenty. The samples were positioned between the grips (effective length = 10mm) and
182 charged until total rupture. The loading speed was set in 0.06 mm/s. Stress-strain curves
183 are recorded and the following parameters were calculated: Young Modulus, Yield
184 stress, elastic deformation, ultimate tensile stress (UTS) and ultimate strain.

185 **2.4.2 Aqueous environment assays**

186 For this series of experiments a sealing recipient (5L capacity) was positioned
187 around the grips of the Bose Electroforce 3230 test instrument. The samples were
188 placed and the recipient filled with water at 4 different temperatures (25, 33, 37 and 40 °
189 C). All tested temperatures are above the PCL glass transition temperature (-60° C)
190 [32]. From this point, traction tests were performed following the same protocol used
191 for traction experiments described above.

192 **2.4.3 Fatigue experiments**

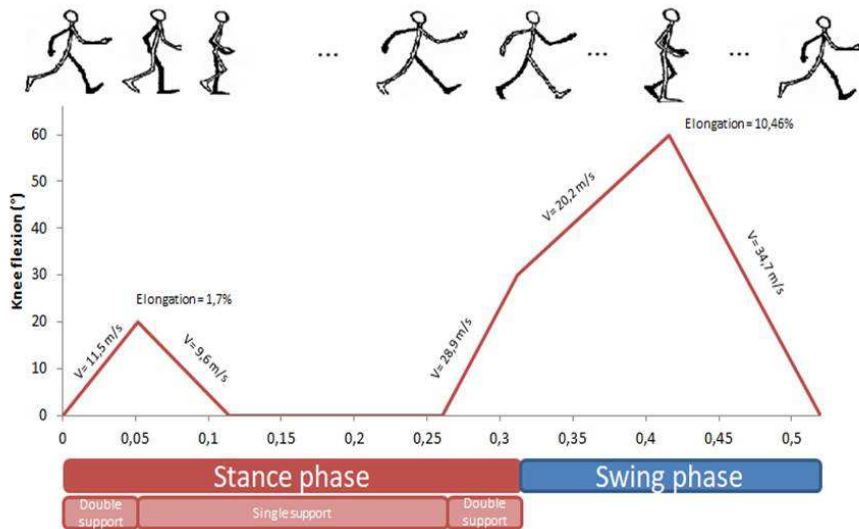
193 The samples were subjected to 1,000,000 sinusoid cycles of charge using the
194 Bose Electroforce 3230 test instrument. The cycles were performed at 3 and 5 Hz with
195 displacement controlled for 10 and 15% of the samples length. The deformation values
196 were established based in the ACL elongations during 90° bending movements (as in the
197 sit motion) and overflexion (120 °) previously reported [33]. The stress/number of
198 cycles curve (S-N curve) was normalized by the initial stress and the rate of resistance
199 loss was evaluated.

200 **2.4.4 Walk Cycle experiments**

201 The Bose Electroforce 3230 test instrument was programed to perform the
202 following protocol, aiming to emulate the movements of ligament elongation and
203 relaxation during the walk phases by considering an average pace of 1.528 m/s or 5.5

204 km/h [33]. The march was divided in two main phases: the support or stance phase
 205 (60% of the time or 0.312 seconds) and the oscillating or swing phase (40% of the cycle
 206 or 0.208 seconds). The support phase was divided into 3 stages: during the double
 207 reception support phase (0.052 seconds) the samples were stretched by 1.7%, then for
 208 the single-limb support phase the the samples were relaxed by 1.7% (0.0624 seconds) to
 209 remain unsolicited for 0.1456 seconds and then stretched by 4.3% during the double
 210 propulsion phase (0.052 seconds). The oscillating phase was divided into two stages
 211 (0.104 seconds each): in the first, the samples were stretched by 5.84% and then relaxed
 212 by 10.46% for the second one.

213 Fig 1 shows the phases of the walk with their duration and the elongation of the
 214 ACL according to the degree of flexion of the knee [33]. After simulating this cycle for
 215 30 min, the poly(caprolactone) samples were subjected to a tensile test loading at 0.06
 216 mm/s until total rupture.



217
 218 **Fig 1.** Schematic representation of ACL's elongation during the phases of gait. Inspired by Mark Dutton [35].

219

220 **2.4.5 Statistical analysis**

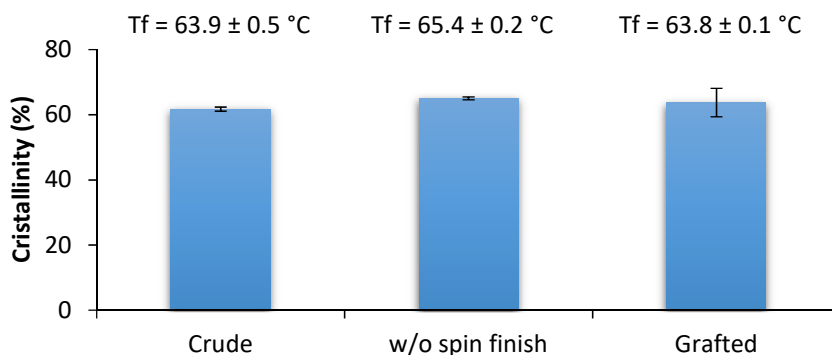
221 All physical-chemical and biological characterization assays were performed in
222 triplicate (n=3), mechanical characterization assays were performed at least 5 samples
223 for each studied group. All presented statistical differences between mean values were
224 calculated with One-Way ANOVA test using excel software ($\alpha < 0.05$).

225 3. Results and discussion

226 3.1 Materials Characterization

227 The TB assay confirmed the PNaSS grafting of poly(ϵ -caprolactone) fibers, as
228 well as the negligible value of non-specific stain absorption for non-grafted samples.
229 The values of 142.9 ± 0.8 nmol/cm² for grafted fibers against 10.1 ± 0.1 nmol/cm² for
230 control reaffirm the effectiveness of the surface treatment.

231 The DSC thermograms revealed no remarkable changes in the thermal properties
232 of the fibers over the treatment. As can be seen in Fig 2 a small increase of crystallinity
233 and fusion temperature (Tf) can be verified in fibers after spin finish removal, however
234 it was not statistically relevant since the variation approximates the values to the same.
235 In addition, the crystallinity increases after thermal grafting treatment.



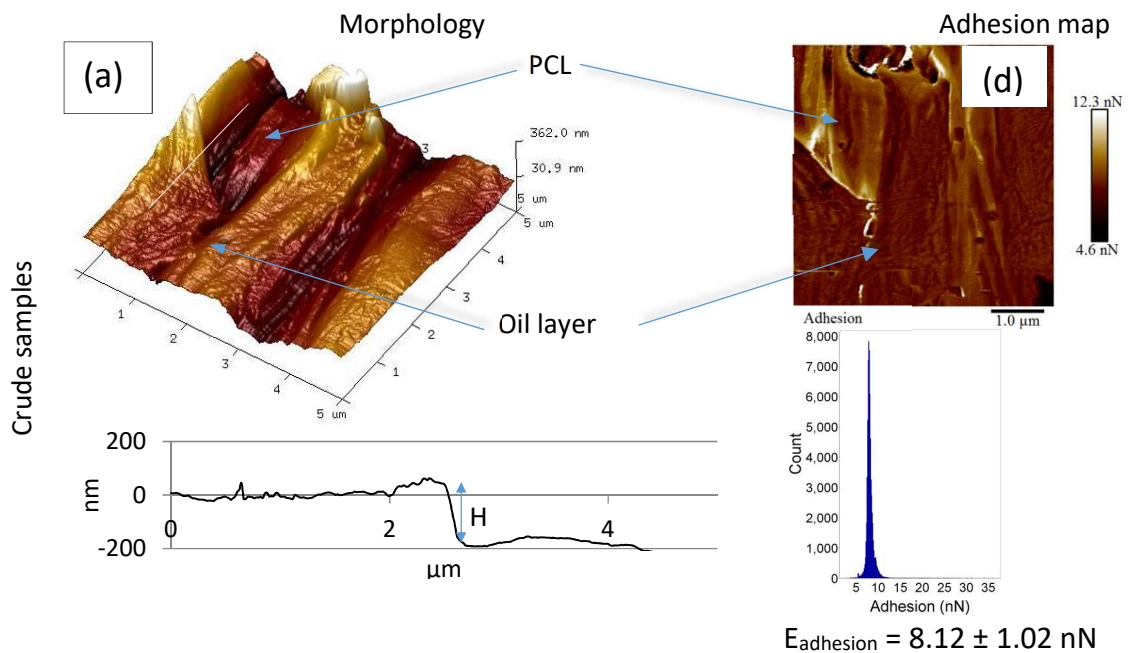
236

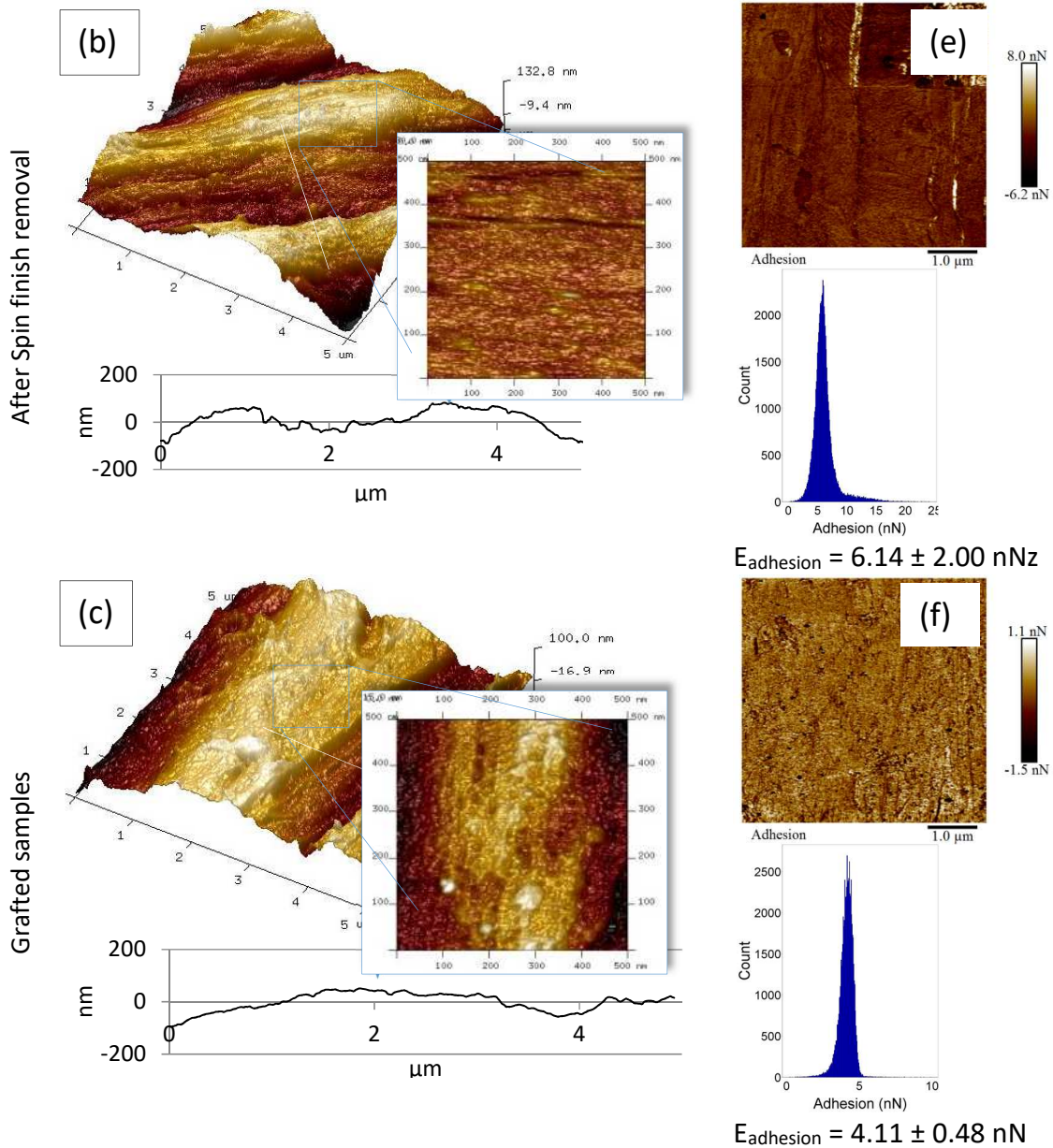
237 **Fig 2.** Crystallinity and fusion temperature of as received, after spin finish removal and grafted poly(ϵ -caprolactone)
238 samples (n=3, $\alpha < 0.05$).

239 The surfaces of fibers were scanned at $5 \mu\text{m} \times 5 \mu\text{m}$ (25°C , $f = 0.5\text{Hz}$, in the air). Fig 3a
240 shows a layer of industrial spinning lubricant over crude samples. This layer has from
241 120 to 180 nm thickness. After spin finish removal and thermal grafting no oil was

242 found over the surfaces and a fibril structure can be noted (Fig. 3b and 3c zoom). No
 243 statically difference was observed in the roughness of the studied groups.
 244 The Fig. 3d, 3e and 3f show the adhesion map between the tip and the fibers. A
 245 reduction of the adhesion force was verified after each step of the treatment: 24%
 246 average reduction after SFR (from 8.12 to 6.14 nN) and 49% after thermal grafting
 247 (from 8.12 to 4.11 nN). The residual stress on polymeric surfaces has been reported to
 248 increase the contact force between tip and material [36]. In the case of the PCL fibers
 249 the residual stress can be alleviated by the temperature of the spin-finish removal and
 250 grafting, decreasing therefore the adhesion force.

251





252

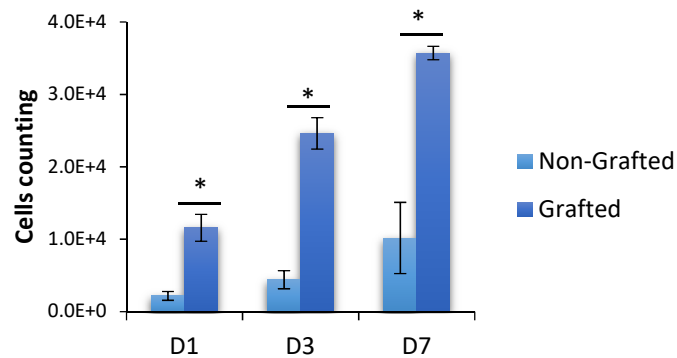
253 **Fig 3.** Topography of polycaprolactone fiber at 5x5 μm scanning area. (a) crude poly(ε-caprolactone) fiber; (b)
 254 poly(ε-caprolactone) after spin finish removal; (c) PNaSS grafted poly(ε-caprolactone) fiber. The “zoom in” of the
 255 images b and c cover 500x500 nm area. The Adhesion map, adhesion force distribution and values (3 different over 3
 256 samples per group) for crude samples, after spin finish removal and PNaSS grafted are showed in figure 3d, 3e and
 257 3f respectively .

258

259 3.2 Cell culture

260 The cells counting showed the influence of the PNaSS presence on cell
 261 proliferation over the poly(ε-caprolactone) fibers. As seen in the Fig. 4, after 24 hours
 262 of cell culture a significant superiority on the cell number was verified for grafted

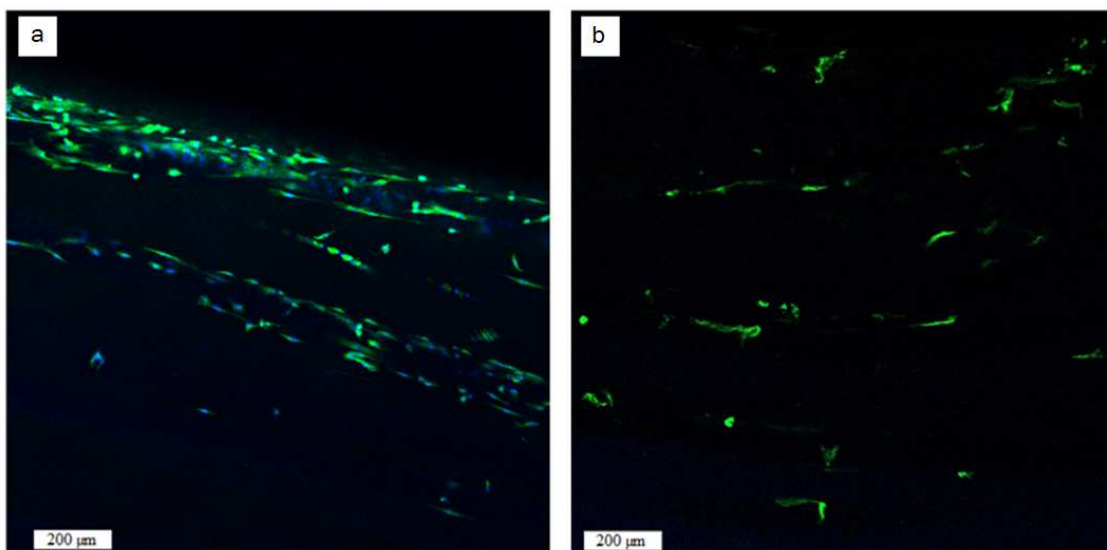
263 samples against non-grafted surfaces. This trend was confirmed for days 3 and 7 with;
264 at least, three times more cells for the grafted group when compared to the non-grafted.



265

266 **Fig 4.** s(ACL) proliferation over grafted and non-grafted samples after 1, 3 and 7 days of culture (n=3, $\alpha < 0.05$).

267 The fluorescence images (Fig 5) confirm the positive influence of the PNaSS on
268 cell behavior after 24 hours of cell culture. Besides the superior number, a higher cell
269 spreading was also verified on grafted sample (Fig 5A).



270

271 **Fig 5.** Fluorescence microscope images of s(ACL) primary cells on a) grafted and b) non-grafted poly(ϵ -
272 caprolactone) samples.

273 As matter of fact, the PNaSS has been linked to early signaling of important
274 adhesion and spreading pathways (FAK phosphorylation and MAPK cascade) [24]
275 creating a favorable environment to cells development. These preliminary results of cell

276 culture support this statement and ratify the previously verified distinguished cell
 277 attachment on grafted poly(ϵ -caprolactone) [25, 26, 29, 37].

278 3.3 Mechanical Properties

279 3.3.1 Influence of the grafting process on the mechanical characteristics of poly(ϵ - 280 caprolactone) bundles

281 Young modulus (E), elastic strain (ϵ), Yield Stress, ultimate tensile stress (UTS)
 282 and ultimate strain were determined for poly(ϵ -caprolactone) crude bundles and after
 283 spin finish removal and pNaSS thermal grafting. Table 1 summarizes the results of the
 284 fibers for different conditions.

285 **Table 1.** Mechanical data obtained from traction experiments (n = 5) and reference native ACL values [5].

	Young's Modulus (MPa)	Yield Stress (MPa)	Elastic Strain (%)	Ultimate Tensile Stress (MPa)	Ultimate Strain (%)
Crude bundles	961 ± 49	312 ± 11	21 ± 2	354 ± 29	80 ± 12
Bundles without spin finish	789 ± 60	265 ± 21	28 ± 1	297 ± 32	96 ± 12
Bundles after thermal grafting	717 ± 64	254 ± 22	31 ± 1	280 ± 17	76 ± 14
Native ACL (male/female)	163/149	-	10-15/10-15	36.4/31.5	36/35

286

287 Crude poly(ϵ -caprolactone) bundles showed the highest value for Young
 288 modulus (961 ± 49 MPa) with an elasticity of 21 ± 2% and a UTS of 354 ± 29 MPa.

289 After spin finish removal both Young modulus and the UTS decreased, 18%
 290 (789 ± 60 MPa) and 16% (297 ± 32 MPa) respectively, while the elastic deformation
 291 was 33% higher (28 ± 1%).

292 The same tendency was verified and amplified after the PNaSS grafting process.
 293 The Young Modulus was 25% smaller than the original value (717 ± 64) and a decrease
 294 of 21% for UTS (280 ± 17) was verified. As expected, the elastic strain was higher (31

295 $\pm 1\%$) than for crude PCL. The reduction of the Young modulus can be explained by the
 296 heating performed in both removal of spin finish components and thermal grafting. The
 297 wires fabrication process include sudden temperature variation, which was reported as
 298 responsible for create residual tension on polymers [38, 39] and increasing the Young's
 299 modulus. Although no significant changes were verified in the crystallinity after spin
 300 finish removal and grafting (Fig. 2), as seen in the adhesion energy decrease verified by
 301 AFM analysis (see Fig. 3), the associated thermal treatments (around 47% of the
 302 melting temperature for 6 h and 70% of the melting temperature for 1 h respectively)
 303 could act as an annealing step [40] of the PCL bundles, relieving the residual tensions
 304 and softening the material.

305 **3.3.2 Environmental influence on the mechanical characteristics of the PNaSS** 306 **grafted and non-grafted poly(ϵ -caprolactone) bundles**

307 Considering that the knee joint reaches temperatures between 33°C and 36.7°C
 308 after 1h walking [41] the samples were immersed in water at 4 different temperature
 309 conditions: room temperature (25°C), 33, 37 and 40 °C (as severe inflammation
 310 situation). The results were compared to assays performed in air at room temperature
 311 (Table 2).

312 **Table 2.** Mechanical data obtained from traction experiments in aqueous media (n=5).

	Temperature	Young's M (MPa)	Elastic strain (%)	UTS (MPa)
Crudes bundles	Air (room temp)	961 \pm 49	21 \pm 2	353 \pm 29
	25	923 \pm 32	25 \pm 2	323 \pm 29
	33	901 \pm 41	27 \pm 3	276 \pm 12
	37	843 \pm 29	26 \pm 1	329 \pm 37
	40	798 \pm 67	27 \pm 1	313 \pm 22
Bundles after thermal grafting	Air (room temp)	717 \pm 64	31 \pm 1	280 \pm 17
	25	878 \pm 99	26 \pm 4	301 \pm 15
	33	933 \pm 39	24 \pm 2	351 \pm 6
	37	887 \pm 79	24 \pm 2	318 \pm 30
	40	618 \pm 83	28 \pm 1	279 \pm 14

313

314 These results confirm the viscoelastic behavior of the polymer [42] since the
315 increase of the assays temperature will induce a reduction of Young modulus. The rise
316 of the temperature weak the secondary bonds between the molecules and, by
317 consequence, decrease the stress required to deform the material. For crude material the
318 values varies almost linearly from 961 ± 49 to 798 ± 67 MPa (18 % reduction) at the
319 highest temperature (40 °C). For the elastic strain and UTS, the environment seems to
320 have a stronger influence than the temperature itself. From the lowest temperature
321 (25°C) the elastic deformation was slightly increased and UTS decreased. Both
322 properties oscillate between this temperature and 40 °C, finishing respectively at 27 ± 1
323 % (26 % higher) and 313 ± 22 MPa (11% lower).

324 Oppositely, when introduced in aqueous environment, PNaSS grafted samples
325 experienced an increase of the Young modulus and a reduction of the elastic strain.
326 However, this behavior, along with the UTS improvement, is not proportional as the
327 temperature increased. The tendency seems to change after 33 °C, finishing with Young
328 modulus and UTS both lower than at 25 °C and elastic strain slightly increased.

329 In general, it can be noted that for all three parameters the aqueous environment
330 mitigates the differences between crude and PNaSS grafted samples and, most
331 important, even at the most extreme conditions the surface modification will not menace
332 the mechanical performance. The reversible deformation required for ACL for
333 prosthesis applications is between 10 and 13% (the average strain of the ACL during
334 walking) [43] and the UTS 38 MPa [44]. Even in the worst-case scenario the modified
335 samples can easily respond these requirements with twice the strain and UTS 8 times
336 higher than the native ACL.

337 **3.3.3 Fatigue experiments**

338 The final stress was determined for crude poly(ϵ -caprolactone) bundles, bundles
 339 after spin finish removal and PNaSS grafting (Table 3 and Fig 6).

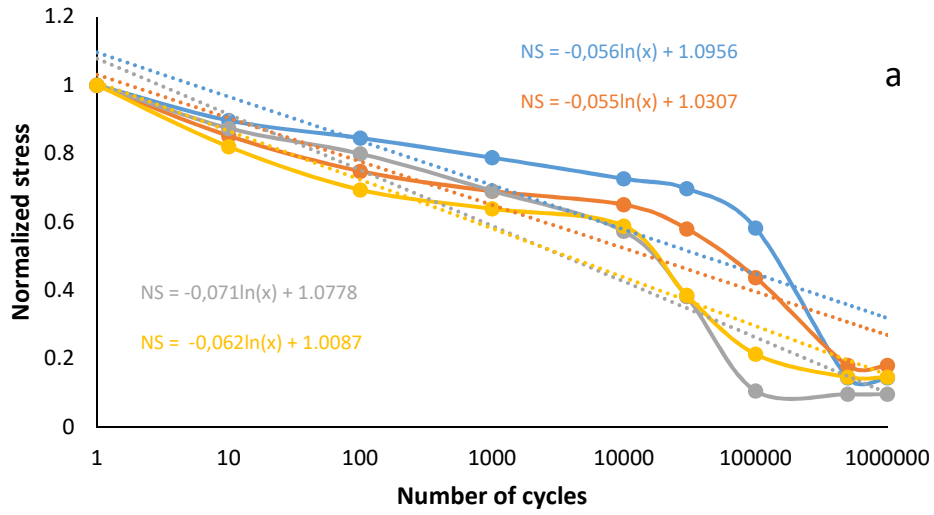
340 **Table 3.** Mechanical data obtained from fatigue experiments (n=5).

Deformation	Final stress (%)			
	10%		15%	
Frequency	3 Hz	5 Hz	3 Hz	5 Hz
Crude bundles	15 ± 4	18 ± 0	10 ± 1	13 ± 4
Bundles without spin finish	33 ± 10	36 ± 10	20 ± 6	35 ± 14
Bundles after thermal grafting	49 ± 12	47 ± 13	13 ± 4	26 ± 22

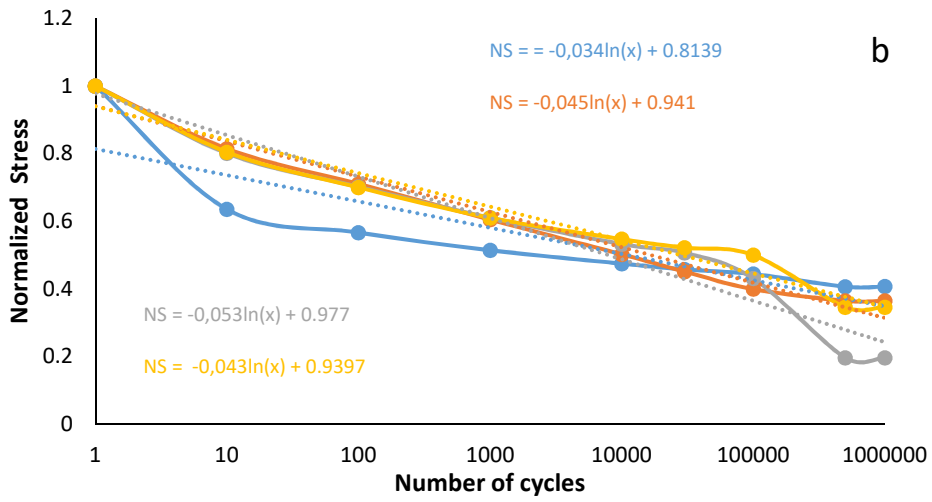
341
 342 As exhibited at Fig 6, crude samples submitted to the same deformation present
 343 similar behavior along with the assay, suggesting that this parameter has a stronger
 344 influence in the resistance loss than the frequency of the movement, which is natural as
 345 the prosthesis, would be more damaged with a 120 ° bending motion than a 90 °
 346 bending. At the same time, these samples had an important loss of resistance
 347 throughout the test, finishing at 10 to 18% of the initial value.

348 In the case of fibers without spin finish components, samples had a lower loss of
 349 resistance (conserving between 20 and 36% of the initial resistance) and thermal grafted
 350 bundles presented the higher remaining resistance of the study (between 13 and 49% of
 351 the initial value). Once again the deformation length showed a strong influence on the
 352 results, however, the difference was much more pronounced for PNaSS grafted samples
 353 than for crude bundles.

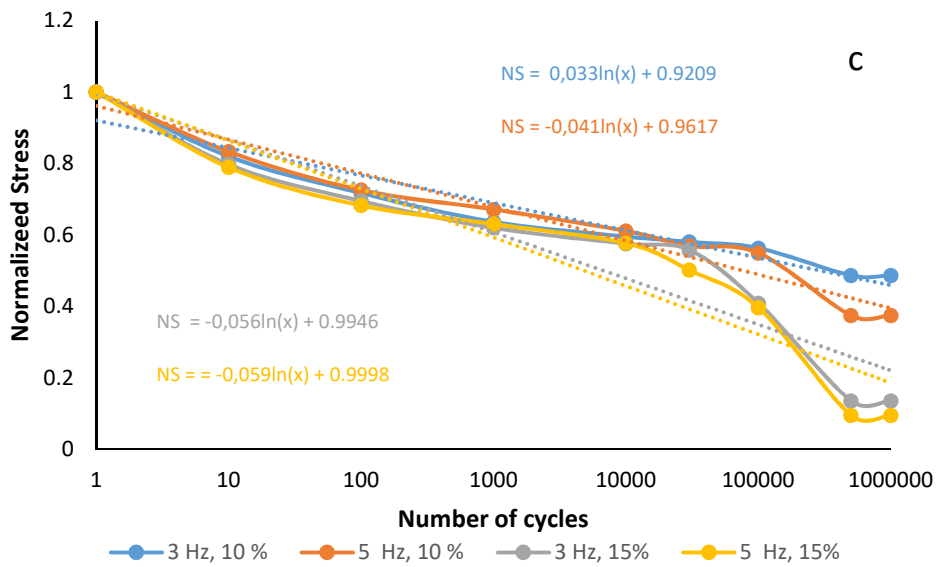
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357

358 **Fig 6.** Normalized S-N curves for fatigue experiments on a) crude poly(ϵ -caprolactone), b) poly(ϵ -caprolactone) after
 359 spin finish removal and c) grafted poly(ϵ -caprolactone) fibers (n=5).

360 Considering the results as a percentage, it's possible to convert these values on
 361 remaining stress for the samples after 1000, 100.000 and 1 million cycles. The values of
 362 stress (αf) for these three groups are exposed in the Table 4.

363 **Table 4.** Maximum and minimum values of the remaining stress for crude PCL samples, PCL after spin finish
 364 removal and thermal grafted PCL after 1000, 100.000 and 1 million cycles.

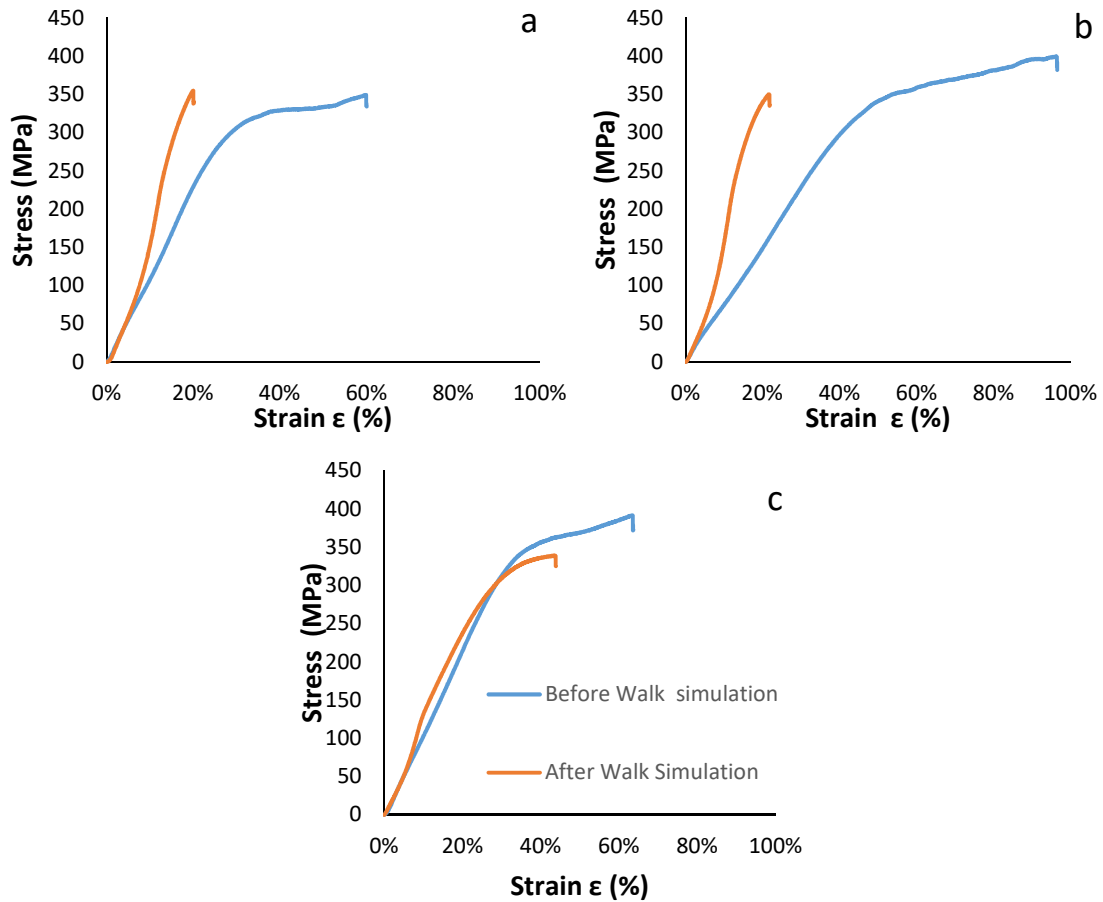
Number of cycles	Af (MPa)		
	1000	100.000	1 million
Crude bundles	276 $\leq \alpha f \leq$ 223	205 $\leq \alpha f \leq$ 39	60 $\leq \alpha f \leq$ 35
Bundles without spin finish	181 $\leq \alpha f \leq$ 151	149 $\leq \alpha f \leq$ 116	107 $\leq \alpha f \leq$ 56
Bundles after thermal grafting	187 $\leq \alpha f \leq$ 173	157 $\leq \alpha f \leq$ 112	134 $\leq \alpha f \leq$ 36

365

366 With the aforementioned UTS value of natural ligament in mind, all the studied
 367 conditions will present at least a resistance similar to the human ACL, even in the
 368 extreme case of 1million cycles. However, in a more practical scenario a valuable
 369 information can be drawn from the evolution of the fatigue behavior. Even though the
 370 crude samples presents higher stress values after the first 1000 cycles, they suffer the
 371 stronger reduction compared to the other groups, reaching very low stress levels already
 372 after 100.000 cycles. Once again, this different behavior between crude samples and
 373 after “thermal treatments” can be linked to reduction of the residual stress, which was
 374 reported to accelerate fatigue damage [45].

375 3.3.4 Walk Cycle experiments

376 After a 30 minutes cycle of walk the PCL samples were subject to tensile tests,
 377 the main mechanical properties were determined and compared to results obtained
 378 without walking simulation. The figure 7 shows the typical aspect of the curves and the
 379 results are detailed in table 5.



380

381

382 **Fig 7.** Stress-strain curves for tensile experiments before and after simulated walk for a) crude, b) after spin finish
 383 removal and c) grafted poly(ϵ -caprolactone) fibers .

384 The general behavior of the samples was typical of semi-crystalline polyester with both
 385 linear and non-linear elastic region [29] suffering strain-hardening in all three
 386 conditions. This phenomenon takes place during the walking simulation test. In order to
 387 keep the pace of the walking, during the simulation the samples were stretched with a
 388 much higher strain rate than used during the tensile tests inducing an increase of the
 389 Young modulus of the samples [46].

390 **Table 5.** Mechanical data obtained from walk cycle experiments (n=5).

		Young modulus (MPa)	Elastic strain (%)	Ultimate tensile strain (MPa)
Crude bundles	Before walk	961 ± 49	21 ± 2	353 ± 29
	After walk	1361 ± 56	12 ± 1	340 ± 29
Bundles without spin finish	Before walk	789 ± 60	28 ± 1	297 ± 32
	After walk	1220 ± 136	12 ± 0	360 ± 17

Bundles after thermal grafting	Before walk	717 ± 64	31 ± 1	280 ± 17
	After walk		1147 ± 56	12 ± 1

391

392 Crude bundle's Young modulus varies from 961 to 1361 MPa (41% increase)
 393 and the elastic strain from 21 to 12 (43% reduction). It's important to notice that in the
 394 curve after walk (Fig 7a) the UTS value was not significantly changed, however the
 395 rupture occurs just after the elastic zone, showing almost no plastic deformation.

396 For bundles without spin finish, the Young modulus has an increase from 789 to
 397 1220 (54% increase) and the elastic strain decreased from 28 to 12 (55% decrease).
 398 Once again almost no plastic zone was verified (Fig 7b).

399 Bundles after thermal grafting showed similar behavior, with Young modulus
 400 increasing 59% and elastic strain decreasing 61%. Yet, contrarily to the two previous
 401 conditions, a small plastic zone can be noticed before the rupture (Fig 7c) and at 300
 402 MPa the sample showed almost 40% deformation without rupture. Naturally, the
 403 material should operate in the elastic zone, however, since the elastic strain of all
 404 conditions are close to the minimum required such toughness improvement could be an
 405 interesting edge against prosthesis failure.

406 **4. Conclusion**

407 The study aimed to demonstrate that poly(caprolactone) fibers can present
 408 suitable mechanical properties to native ACL replacement after surface
 409 functionalization. This goal was achieved by different mechanical assays, trying to
 410 approach the conditions of the natural ligament daily efforts. The results indicate that, in
 411 addition to the already recognized biological response enhancement brought by this
 412 procedure, the "thermal treatment" inherent to this functionalization technique create a
 413 desirable side effect that can improve the ability of the PCL fibers to adapt to different

414 environments and mechanical demands. Therefore, the combination of the results
415 justifies the interest of the choice of poly(ϵ -caprolactone) for the elaboration of ligament
416 prosthesis with appropriate mechanical behavior

417 **Conflict of interest**

418 The authors declare that they have no known competing financial interests or personal
419 relationships that could have appeared to influence the work reported in this paper.

420 **Credit authorship contribution statement**

421 Andre Rangel: Investigation, Formal analysis, Methodology, Writing - original draft.
422 Laila Colaço: Investigation, Writing - original draft. Ngoc Tuan Nguyen: Investigation,
423 Formal analysis. Jean-François Grosset: Conceptualization, Formal analysis,
424 Methodology. Christophe Egles: Conceptualization, Formal analysis, Resources,
425 Supervision, Writing - review & editing. Veronique Migonney: Conceptualization,
426 Formal analysis, Funding acquisition, Methodology, Project administration,
427 Supervision, Validation, Writing - review & editing.

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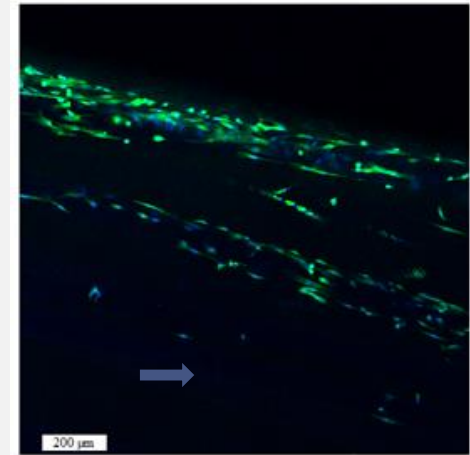
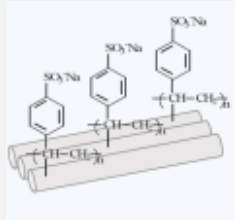
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Biodegradable synthetic ligament

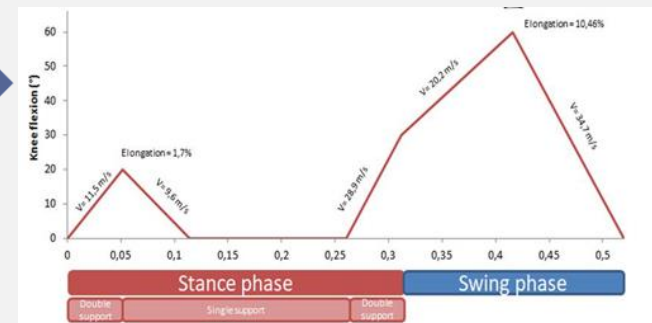


ACL rupture

bioactive polymer grafting



cell colonization



mechanical properties/daily activities