Different real-time degradation scenarios of functionalized poly(ϵ-caprolactone) for biomedical applications

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ARTICLE

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Different real-time degradation scenarios of functionalized poly(ε-caprolactone) for biomedical applications

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Abstract

Anterior cruciate ligament (ACL) ruptures are a much-commented injury as it can end the season or even career of professional athletes. However, the recovery of a patient from the general population is no less painful during the long period required by current treatments. Artificial ligaments could improve this healing, yet, orthopedic surgeons are still cautious about permanent ACL implants. Therefore, combining biodegradation and bioactivity could be a key feature for the popularization of these devices. This study aim at evaluating the real-time degradation of poly(ε-caprolactone) (PCL) grafted with the bioactive polymer sodium polystyrene sulfonate in different scenarios. PCL physical–chemical properties were evaluated before and after degradation. In addition, in vitro experiments were realized to confirm the long term influence of the grafting on cell response. Altogether, we were able to show different degradations scenarios, enabling to study the impact of degradation environment on degradation mode and rate of functionalized PCL.

KEYWORDS

biodegradable, biomaterials, biomedical applications, degradation, functionalization of polymers

1 | INTRODUCTION

Through the last decades, anterior cruciate ligament (ACL) injuries have been a recurrent medical issue in the general population with an incident rate of 1/3000.[1] Such injuries, habitually caused by sudden hyperextension or twist of the knee, are also very common in professional sports. Since the ACL plays a fundamental role in stabilizing the joint after the rupture patients usually have their movement limited by pain or/and instability of the knee. Even worst, due to its intra-articular location and poor vascularity, the ACL does not heal spontaneously[2]; the injury can harm the practice of recreational or professional activities, and ultimately evolve to meniscal lesions and cause arthritic degradation of the knee. Face of it, a surgical procedure is often recommended for ACL reconstruction.

The use of artificial materials for this application was set back by the problems related to both biocompatibility and incompatible mechanical behavior of the first generation of prostheses, particularly carbon and PTFE based.[3]

The currently "gold standard" for ACL replacement is the autograft from autologous tendon,[4] however, the limitations of this technique are not negligible, especially the morbidity of the harvesting site and the prolonged recovery period.[2] To fight this problem, our team has worked on the surface functionalization of non-toxic, mechanical compatible synthetic ligaments made of poly (ethylene terephthalate) (PET).[5–7]

The biofunctionalization with poly(sodium styrene sulfonate) (pNaSS) aims to modulate the cell attachment and proliferation by having a glycosaminoglycan-like polymer grafted on the surface. The presence of the pNaSS can create active sites for extracellular matrix binding proteins interaction, improving the cell response.[8] This effect was verified on different surfaces[9–11] for both in vitro and in vivo assays.[12,13] Yet, PET ligaments still suffer from the echoes of previously failed materials and the unsure position of healthcare professionals regarding a permanent prosthesis for this application.[14] Therefore, the use of a biocompatible biodegradable polymer came as an ideal answer to the actual scenario.

Poly(ϵ-caprolactone) (PCL) is a low-cost, biodegradable, and biocompatible aliphatic polyester with a large range of applications in the biomedical field.[15] The main advantage of the PCL over other degradable polymers is the absence of acid degradation products and the long degradation time.[16] The low degradation ratio can be bonded to the presence of five CH_2 groups on the polymer's structure, making it hydrophobic and less vulnerable to hydrolysis.[17]

While this low degradation rate is a clear advantage if we are looking for a material that will last long enough to allow the new ligament to growth, the hydrophobicity can backfire on the cell development,[18] and then the surface functionalization becomes vital for a favorable outcome. Equally, the inflammatory events that could happen on the knee region are often correlated to the rise of proteolytic enzymes amount in the human synovial fluid[19] and the mechanism of the PCL degradation could be highly impacted by the presence of such enzymes.[20,21] Therefore, a study of the real-time degradation of the PCL on physiological and enzymatic conditions is a key point for the future application of this material as an ACL replacement and the effect of the surface functionalization by pNaSS on this process represents a completely unexplored factor.

Therefore, this article aimed at following the surface and structural degradation of medical-grade PCL at physiological conditions and its influence on the physical–chemical, mechanical and biological properties of the samples for as long as one and a half years. The results showed the impact of the surface treatment and degradation environment in the degradation mode and rate but also confirmed the biological performance of the pNaSS grafted can resist to in vitro degradation.

2 | EXPERIMENTAL

2.1 | Samples grafting

Medical-grade PCL pellets (Corbion Purasorb PC12, Netherlands) were dissolved in dichloromethane (40% wt/vol) and spin-coated at 1000 rpm for 30 s. Dried films were cut in 14 mm diameter round samples and stored at 4°C. Medical-grade PCL fibers (diameter = 100 μm) were kindly supplied by MDB Texinov (France). Bundles of 20 fibers were handmade and exposed to soxhlet extraction in ethyl ether for 4 h to remove spin finishing components, dried at room temperature and stored at 4°C. From this point the samples were divided in two groups: half was kept as virgin PCL films and fibers to be used as control group for this study (NG group) and the other half was submitted to pNaSS grafting using the "grafting from" technique (G group). The grafted surfaces were activated by ozonation (BMT 802 N ACW, oxygen flow rate of 0.6 L min^{-1}) in distilled water at 30°C (20 min for films and 10 min for fibers bundles) and quickly transferred to a balloon containing NaSS (Sigma–Aldrich, France) solution (0.7 M). The balloon was then put on an oil bath at 45°C for 1 h. After pNaSS grafting the samples were extensively washed in distilled water, dried under vacuum and stored at 4°C.

2.2 | Degradation study design

The degradation study was conducted for both pNaSS-grafted (G) and non-grafted (NG, control group) PCL films and fibers individually immersed in a vial containing 20 mL of PBS or collagenase solution (50 μg/ml, Type 1 from Clostridium histolyticum, 125 CDU/mg in 0.01 M Tris-base 50 mM, $CaCl_2$ 4 mM and NaCl 0.15 M). The pH of the collagenase solution was adjusted to 7.6 by HCl addition. The vials were closed to avoid evaporation and degradation study was carried out in an incubator at 37°C from 4 to 72 weeks in PBS and for 24 weeks in collagenase. The PBS was renewed every 3 months and the collagenase solution every month to control the solution pH. No acidification of the degradation media was verified over the study. The samples were characterized before and after degradation to evaluate the effect of the environment on the medical-grade PCL.

2.3 | Surface degradation characterization

The morphology of the surfaces was surveilled by scanning electron microscopy (SEM) (Hitachi TM3000 SEM)

and the roughness by Atomic Force Microscopy Atomic (Multimode 8, Bruker) using the NanoScope Analysis 1.5 software and Scanasyst-Air program (V shape tip, k: 0.4 N/m, f_0: 70 kHz). The AFM scanning in the air was performed at room temperature (25°C). The scan size were 5 μm × 5 μm, 1.5 μm × 1.5 μm, and 500 nm × 500 nm (scan rate, f = 0.5 Hz).

The surface wettability was analyzed by water contact angle using a DAS10 goniometer from Kruss. For each measurement a 200 μl droplet was deposed onto the surface and a single image was taken 8 s after contact. The contact angle was measured by the Kruss software from these images.

Fourier transformed infra-red spectroscopy in attenuated total reflection mode (ATR Perkin Elmer Spectrum Two) was used to evaluate the chemical bond changes caused by degradation. A total of 128 scans (4000–400 cm^{-1}, 2 cm^{-1} resolution, diamond crystal) were performed in three different points per sample.

The pNaSS grafting density for crude samples and after 24 weeks of degradation was determined using the Toluidine Blue-O (Roth Sochiel, Germany) colorimetric assay.[22] The samples were immersed in a toluidine blue solution (0.5 mM) and kept at 30°C for 6 h. The samples were washed three times in 1 mM NaOH to remove the exceeding staining and soaked in acetic acid solution (50% in water, vol/vol) for 24 h for decomplexation. A volume of 1 mol of pNaSS complexed 1 mol of staining on the surface. Light absorbance of the resulting solution was measured using a UV/visible spectroscopy (Perkin Elmer Lambda 25 spectrometer, USA) at 630 nm and compared to a standard curve of known toluidine blue concentrations.

2.4 | Structural degradation characterization

The variation of molecular weight (M_n and M_w) and polydispersity was evaluated by Gel permeation chromatography (Shimadzu Prominence instrument LC20AD, Shimadzu RID-10A refractive index detector). Ten milligrams of PCL was dissolved in tetrahydrofuran (Rotisolv CLHP) and eluted through two columns (Phenomenex phenogel). A calibration curve was generated using narrow polydispersity poly(methyl methacrylate) standards. The half-time molecular weight reduction was calculated using the following equation:

$$\ln(Mn) = -kt + \ln(Mn0) \quad (1)$$

The melting enthalpy of the samples was measured by differential scanning calorimetry (DSC 8000 Perkin Elmer, France) under a nitrogen atmosphere. The samples were scanned from −75°C to 100°C at 10°C.min^{-1}. The degree of crystallinity (X_c) was calculated using the following equation:

$$Xc = \Delta Hm/\Delta Hm0 \times 100, \quad (2)$$

where ΔH_m is the melting enthalpy of the evaluated sample and ΔH_{m0} the 100% crystalline PCL melting enthalpy (assumed to be 135.44 $J.g^{-1}$).[23]

2.5 | Mechanical assays

G and NG PCL bundles (length = 10 mm) were submitted to traction assays (Bose Electroforce 3230) before and after degradation. All assays were performed at 0.06 mm/s loading speed and the samples were charged until total rupture. Stress–strain curves were recorded and Young Modulus, elastic deformation and ultimate tensile stress (UTS) were calculated.

2.6 | Cells culture

Anterior cruciate ligaments cells (sACL) were isolated from a 2-year-old female Merino sheep free of degenerative joint disease following with the German legislation on protection of animals and the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication 85–23, Rev. 1985), and as approved by the local governmental animal care committee. Tissues were cut into small pieces of 1 mm^2, washed three times in PBS, and incubated in collagenase solution (0.1% wt/vol, Sigma-Aldrich) at 37°C under 5% CO_2 for 6 h. The suspension was centrifuged 3 min at 1500 RPM. The supernatant was withdrawn and the cells suspended in DMEM (10% fetal bovine serum [FBS], 1% penicillin and 1% L-glutamine) and cultured in T-75 flasks until confluence.

Before in vitro experiments, the PCL samples were extensively washed (3 × 3 h in 1.5 M NaCl solution, 3 × 3 h in 0.15 M NaCl and 3 h in PBS) and sterilized by ethanol 70% vol/vol (20 min washing) and UV irradiation (15 min each side, under a flow hood). The samples were then transferred to well-plates containing 1 mL of FBS free DMEM and kept overnight. Lastly, the samples were transferred to wells containing DMEM media (1% penicillin, 1% L-glutamine, 10% FBS) and stored for 24 h.

The sACL morphology and viability were studied by eosin/hematoxylin staining and MTT assays over G and NG samples before degradation and after 24 weeks in PBS and collagenase solution. All in vitro tested samples

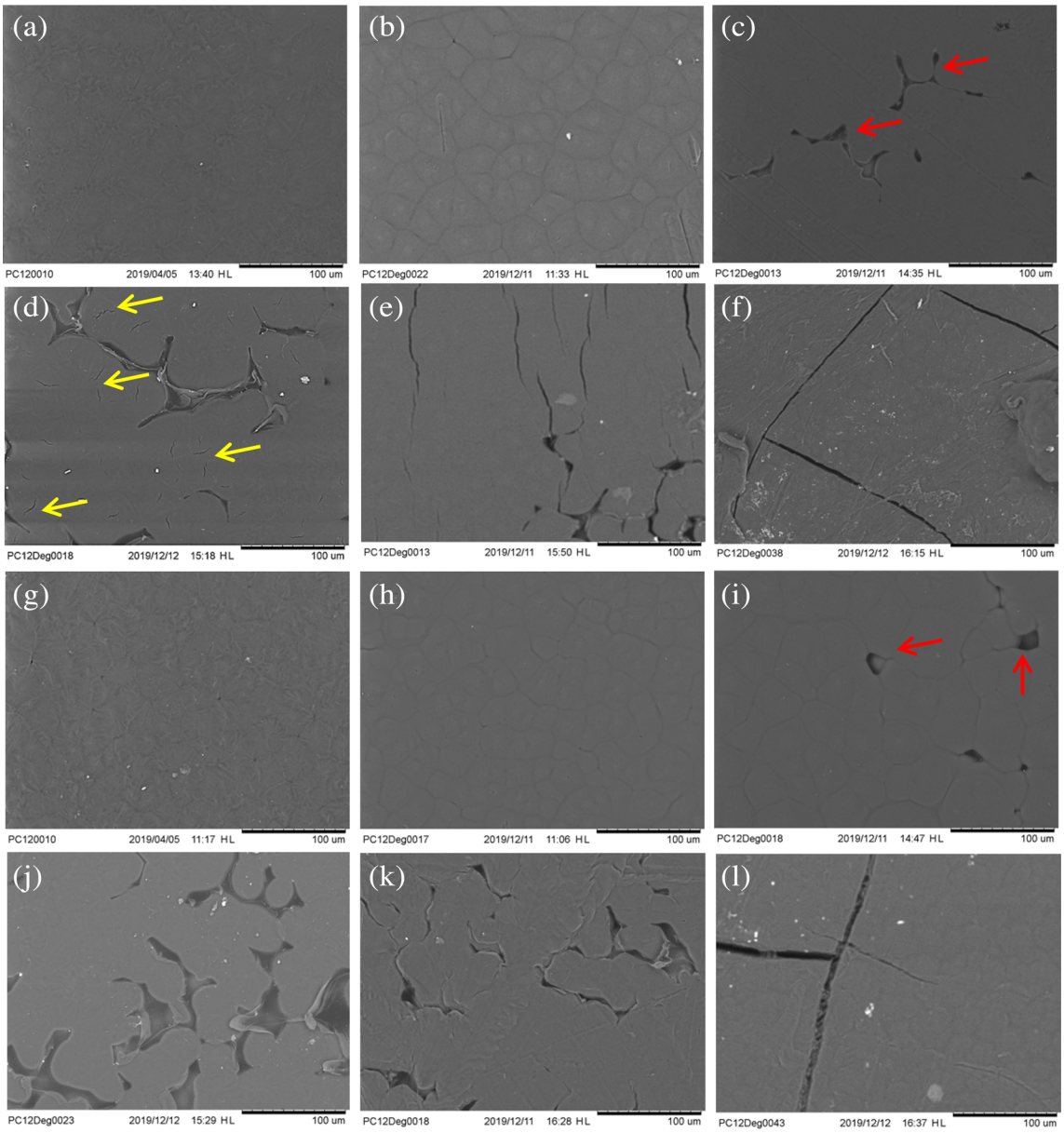

FIGURE 1 Scanning electron microscopy images of medical-grade poly(ε-caprolactone) (PCL) films at different conditions and degradation time points. G samples: (a) non-degraded, (b) 4 weeks of degradation, (c) 12 weeks of degradation, (d) 24 weeks of degradation, (e) 48 weeks of degradation, (f) 72 weeks of degradation. NG samples: (g) non-degraded, (h) 4 weeks of degradation, (i) 12 weeks of degradation, (j) 24 weeks of degradation, (k) 48 weeks of degradation, (l) 72 weeks of degradation [Color figure can be viewed at wileyonlinelibrary.com]

were seeded with 50×10^3 cells/well and cultured at 37°C under 5% CO_2 over the time of the experiment. The culture media was changed every 48 h.

For MTT assays after 24 h of cell culture the samples were rinsed twice in PBS and transferred to a well containing 500 µl of MTT solution in phenol-red-free DMEM (1 mg/ml). The samples were incubated for 4 h at 37°C under 5% CO_2 then rinsed in PBS again and immersed in 350 µl of DMSO for formazan crystals dissolution (10 minutes at room temperature under mechanical stirring). The solutions absorbance were measured by UV spectroscopy (SAFAS MP96 plate reader) at 570 nm and normalized using the following equation:

$$NA = (\mathrm{ODs} - \mathrm{ODb})/(\mathrm{ODc} - \mathrm{ODb}) \quad (3)$$

where ODs is the measured absorbance of the sample, ODb is the absorbance of the blank (DMSO) and ODc is the control absorbance (cells cultured in a well without PCL sample).

The eosin/hematoxylin staining was performed after 1, 3 and 7 days of culture. After the given time, the

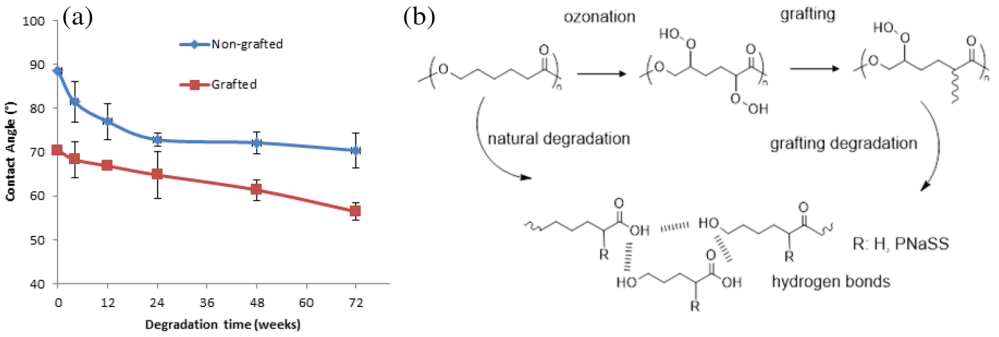

FIGURE 2 (a) Water contact angle measured by the sessile drop method on G and NG medical-grade poly(ε-caprolactone) (PCL) samples and its evolution with degradation time. (b) Scheme of hydrolytic degradation of NG PCL (natural degradation) and G PCL (grafting degradation) [Color figure can be viewed at wileyonlinelibrary.com]

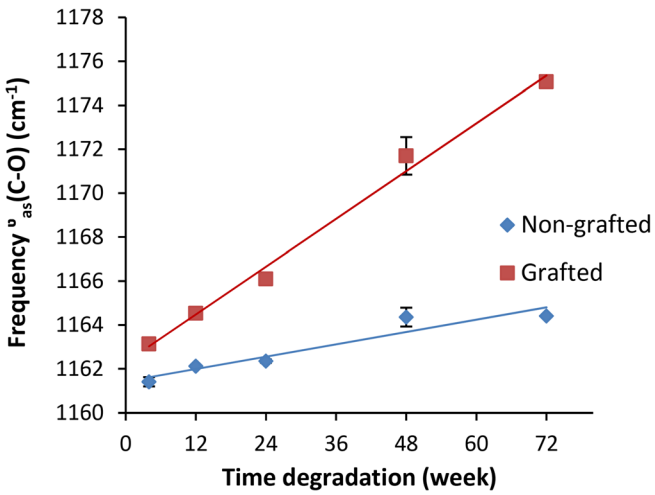

FIGURE 3 Shift of the C—O stretching vibration peak on G and NG samples over 72 weeks of degradation in PBS at 37°C [Color figure can be viewed at wileyonlinelibrary.com]

samples were rinsed twice in PBS and fixed using a 4% formaldehyde solution at room temperature for 30 min. After fixing, the samples where immersed in hematoxylin (Harris modified solution) for 10 min, rinsed in distilled water and 1% HCl solution before washing in water (60°C) for 4 min. The samples were then stained again in hematoxylin for 2.5 min, rinsed in water and stained with eosin Y solution (5 mg/ml) for 1.5 min. Finally, the samples were rinsed in water, allowed to dry at room temperature and stored at 4°C until images.

2.7 | Statistical analysis

Physical–chemical characterization and in vitro experiments were realized in at least three different samples per analysis. All mechanical characterization assays were performed in five samples for each studied group. Statistical differences were calculated with ANOVA test ($p < 0.05$).

3 | RESULTS AND DISCUSSION

3.1 | Medical-grade PCL degradation in PBS

The degradation of medical-grade PCL G and NG films was studied in physiological conditions (PBS at 37°C) for 72 weeks. The surface functionalization has shown an influence on both surface and bulk degradation of the PCL samples.

3.2 | Surface degradation in PBS

As seen in Figure 1, the degradation process started to show an effect in the surface morphology at 12 weeks of degradation for G and NG samples (Figure 1(c) and (i) red arrows). Both conditions showed similar degradation in the amorphous region between the spherulites.[16,24] After this point, the two conditions revealed different behaviors and in addition to the degradation of the amorphous portion, the G samples presented small intra-spherulite cracks at 24 weeks (Figure 1(d), yellow arrows) who evolved to larger cracks at 48 weeks (Figure 1(e)) and generated a brittle surface at 72 weeks (Figure 1(f)). The NG samples kept loosing amorphous matter until the cracks happen suddenly at 72 weeks. However, it can be noticed that the cracks were not complete and some PCL fibrils were still present underneath the surface rupture (Figure 1(l)).

This acceleration of the surface degradation process was attributed to the activation step of the grafting.[25] The slow degradation rate of the PCL, when compared to other polyesters, can be explained by the presence of five CH_2 groups on the polymer's structure.[17] These groups are highly hydrophobic and encumber the hydrolysis of the ester bond, yet the ozonation replaces some of these units with peroxide and radicals making the surface more

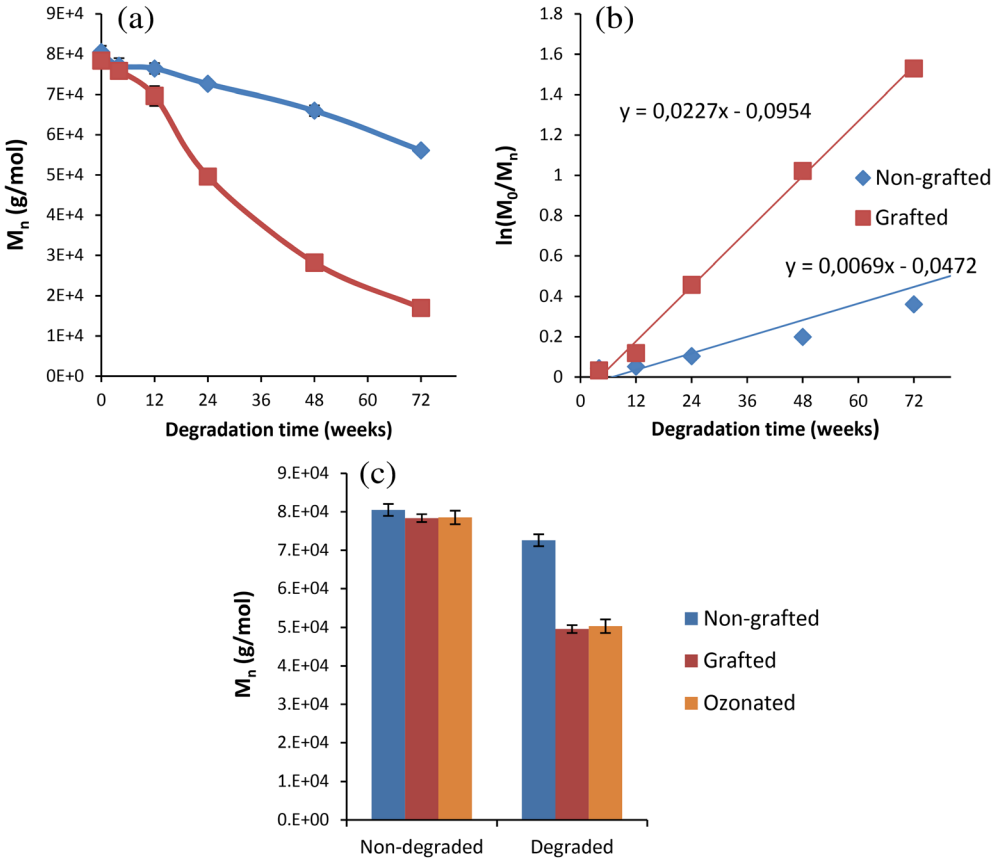

FIGURE 4 Number molecular weight evolution (a), modified Arrhenius plot for half-life molecular weight reduction calculation (b) and number molecular weight variation of G and NG samples compared to ozonated films after 24 weeks of degradation at 37°C in PBS (c) [Color figure can be viewed at wileyonlinelibrary.com]

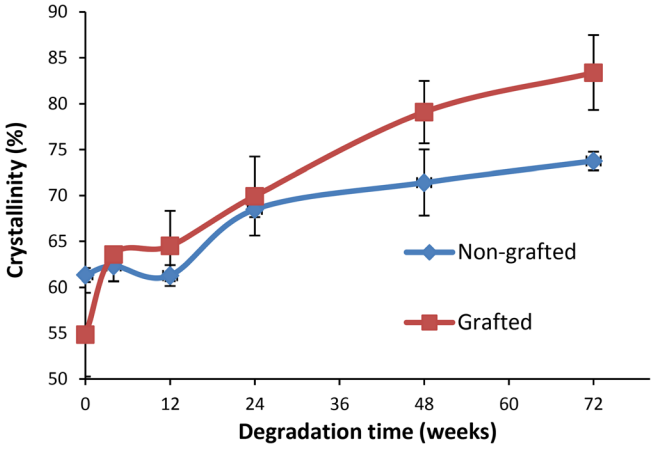

FIGURE 5 Crystallinity rate of G and NG samples degraded in PBS at 37°C for 72 weeks [Color figure can be viewed at wileyonlinelibrary.com]

hydrophilic and turning it more susceptible to hydrolysis to happen, as described in Figure 2.

Moreover, the degradation's products often contain polar groups (COOH/COH), creating an autocatalytic environment propitious to accelerated degradation. One way to evaluate the proportion of COOH/COH generated during degradation is to observe the blue shift of CO vibration (from 1162 to 1176 cm^{-1}). The blue shift corresponds to the high interaction of degraded fraction through hydrogen bonds (Figure 2(b)). H-bonding from COOH/COH impacts the stretching vibration of CO causing the peak to shift.[26,27] As seen in Figure 3 the C—O peak shift from 1663 to 1176 cm^{-1} for G samples while in the NG surfaces it moved from 1661 to 1664 cm^{-1} indicating a more important amount of hydrolysis products on the G group.

3.3 | Structural degradation in PBS

The degradation in physiological conditions has also produced structural modifications in the medical-grade PCL. Figure 4(a) shows the number average molecular weight (M_n) variation for G and NG samples over the 72 weeks of study. Once again, the G samples displayed more intense degradation with 80% of M_n reduction by the end of the experiment against 30% for NG samples. Knowing that both conditions obey the 1rst order Kinect degradation law[28,29] (Figure 4(b)) the Arrhenius equation could be used to calculate the half-time M_n reduction (HL = ln (2)/K). For non-degraded films the molecular weight was expected to be reduced to 50% of the original value after 100 weeks, following values already reported in the literature,[30] however, for G samples this value drops to 30 weeks.

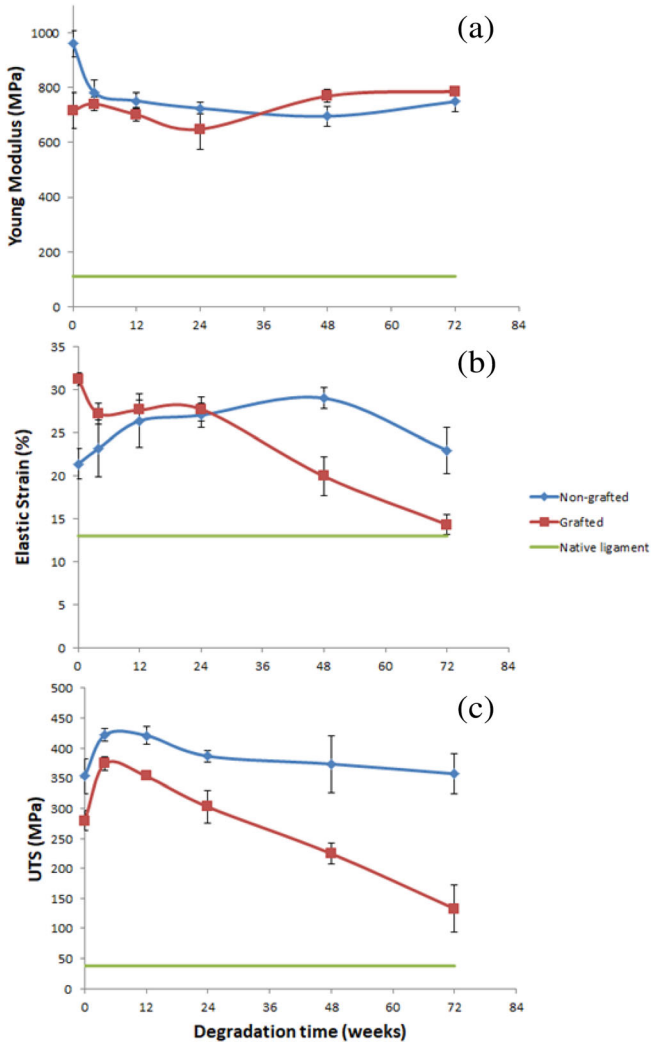

FIGURE 6 Monitoring of Young's modulus (a), elastic deformation (b) and ultimate tensile strength (c) evolution of G and NG samples degraded in PBS at 37°C [Color figure can be viewed at wileyonlinelibrary.com]

To validate the hypothesis that the difference in the degradation rate of the two studied groups was related to the ozonation step of grafting, PCL samples were activated by ozone and incubated in PBS at 37°C. After 24 weeks the samples' molecular weight was assessed and compared to degraded G and NG samples. As shown in Figure 4(c) the variation of G samples' molecular weight was statistically equivalent to the activated surfaces, with a 35% reduction against 10% for NG samples. This result ratifies that the main reason for the accelerated degradation on G samples was the change on surface properties brought by the ozonation and the presence of pNaSS does not impact the degradation rate significantly.

Based on the SEM images, most of this molecular weight reduction started from the amorphous region on the surface then went deeper into the material leading to bulk degradation. The differential scanning calorimetry assays corroborate to this statement. Figure 5 shows a substantial increase of crystallinity degree on G samples (from 54 to 83%), following the most important molecular weight reduction, and consequently amorphous matter loss.

One of the key points of the degradation study was to evaluate how these modifications would affect the mechanical properties of the medical-grade PCL. For doing so Young's modulus (E), the elastic deformation (ϵ) and the UTS were determined from the traction curves of PCL fiber bundles from non-degraded to 72 weeks of degradation (Figure 6).

When the PCL bundles were degraded in PBS at 37°C, the variation of Young's modulus (E) was different between the G and NG samples. For NG fibers, Young's modulus decreased suddenly during the first month then reached a plateau value with a slight increase after 72 weeks. On the other hand for G fibers, E decreased slowly up to 24 weeks then increased regularly up to 72 weeks. This variation of Young's modulus can be associated with two phenomena which take place sequentially: in a first stage the temperature of degradation will weak the secondary bonds of the polymer structure, causing a relaxation of the structure and reducing E. After a certain degradation time, the loss of amorphous matter and the increase of crystallinity induce an increase of the Young's modulus, as expected in semi-crystalline polymers.[31] The elastic deformation (ϵ) varied as expected (Figure 6(b)) taking into account the evolution of Young's modulus. The samples once again showed very different profiles of progression. For NG samples, after an increase of the elastic deformation in the first months of degradation, the values remained statistically stable for several weeks until a decrease at the final time point. The G samples lost elasticity in the first month of degradation then kept the percentage for around 24 weeks before start dropping continually until the end of the experiment. The results were entirely consistent with the parallel increase in Young's Modulus and it is important to note that even at the most degraded condition the value of ϵ was higher than the native ligament.[3,32]

The ultimate tensile strength makes possible to estimate the mechanical resistance of the PCL fibers to a tensile force. For all samples G and NG, the UTS reached a maximum value after 1 month of degradation (Figure 6 (c)). After this first period, different profiles were observed depending on whether the surfaces have been G or not with pNaSS. The NG samples showed a constant and slight decrease of up to 72 weeks. Contrarily, when the samples were G, the decrease after 1 month was linear and significant: the value at 72 weeks reached approximately 50% of the initial value. However, whatever the conditions and degradation time the UTS were always higher than that of the native ligament.

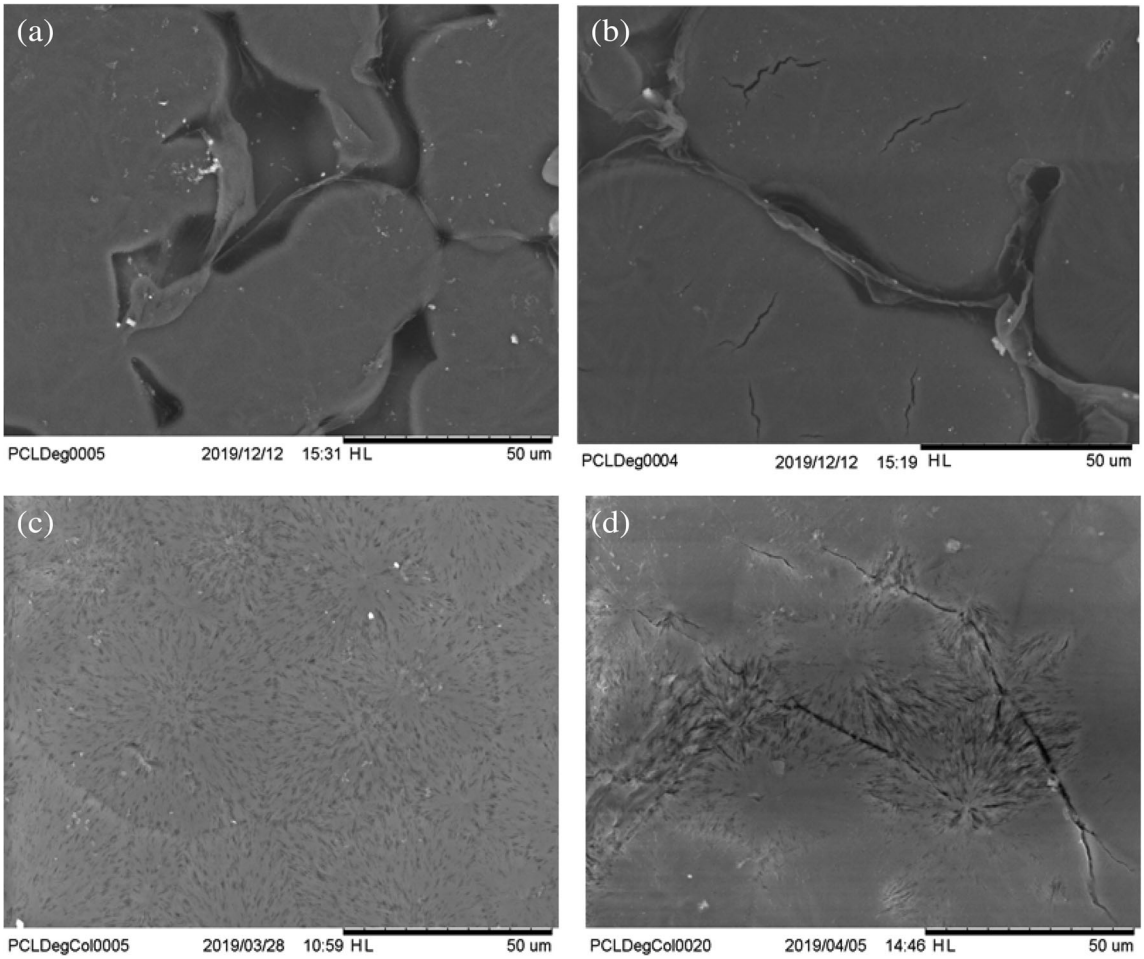

FIGURE 7 Scanning electron microscopy images of poly(ε-caprolactone) (PCL) films NG (a),(c) and G (b),(d) in PBS and collagenase solution, respectively

4 | MEDICAL-GRADE PCL DEGRADATION IN THE ENZYMATIC ENVIRONMENT

4.1 | Surface degradation in collagenase

Figure 7 shows the surfaces of G and NG films degraded at PBS and collagenase solution at 37 °C for 24 weeks.

As reported in the literature,[33] in the enzymatic environment, the degradation of the PCL will take place by surface erosion (Figure 7(c),(d)) in addition to the previously described bulk hydrolytic degradation found after immersion in PBS (Figure 7(a), (b)).

It was possible to note that surface erosion was more widespread in NG samples, nonetheless, in G PCL a combination of the two degradation modes was verified and intra-spherulite cracks caused by hydrolysis were observed. The AFM images (Figure 8) confirm the differences in the shape and size of the defects for G and NG samples.

From Figure 8, the cracks on G PCL degraded in collagenase exhibited an average depth of 88.57 ± 15 nm and a hundred nanometer of length (Figure 8(f)). While, defects by collagenase on the NG PCL surface appeared as more numerous and small dimension dimples (9.01 ± 1.2 nm of mean depth and ∼ 30–40 nm of radius) (Figure 8(e)). This can be explained by the high hydrophobicity of NG PCL before degradation and the tendency of hydrophobic materials to keep the erosion at their surfaces.[34] As seen in Figure 9 the water contact angle of NG films was 20% higher than its G equivalent before degradation.

4.2 | Structural degradation in collagenase

The difference in the degradation mode has also brought modifications to the structural properties of the PCL films. The NG films degraded in collagenase (pure

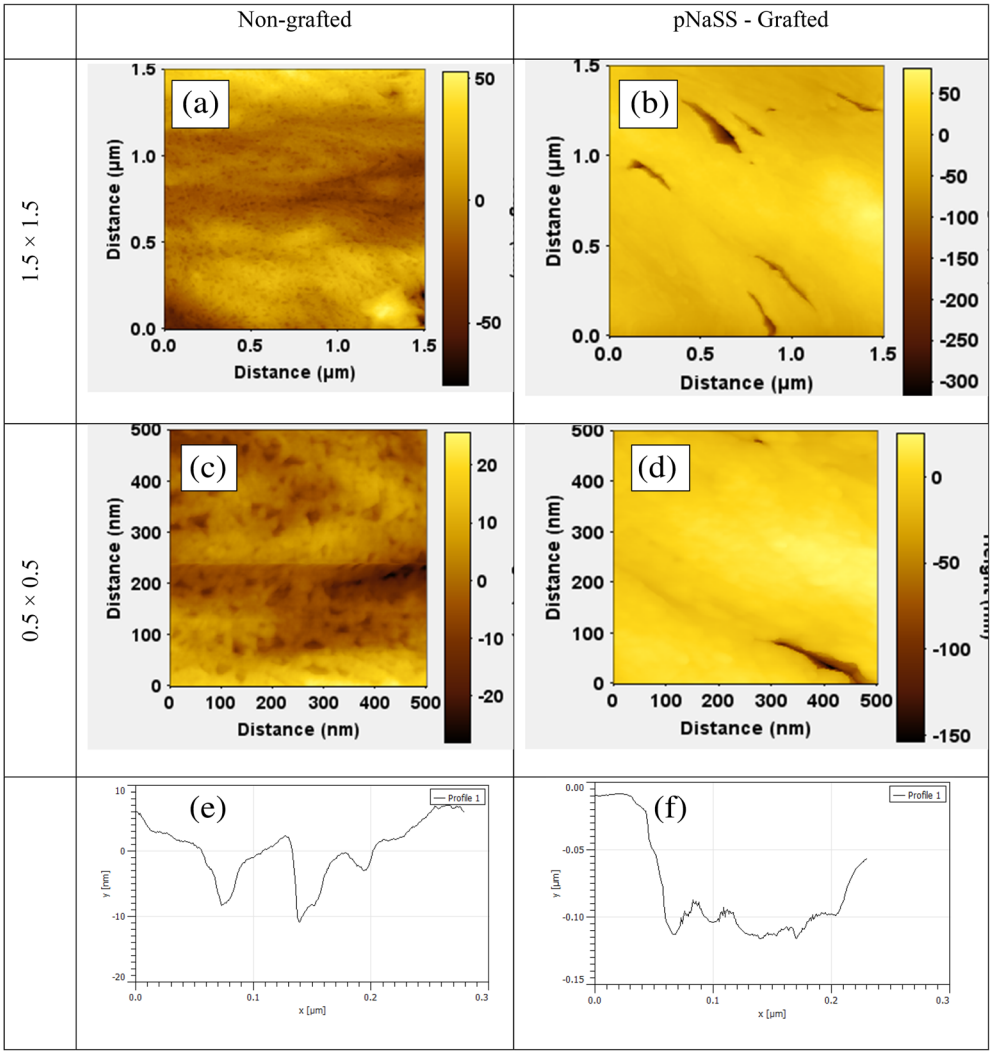

FIGURE 8 AFM images of NG and G poly(ε-caprolactone) (PCL) 24 week degradation. The shapes and size of the generated defects were highly dependent on the surface treatment: (a) topography of non-graft PCL film degraded in col (1.5 μm × 1.5 μm), (b) topography of G PCL film degraded in col (1.5 μm × 1.5 μm), (c) topography of non-graft PCL film degraded in col (500 nm × 500 nm), (d) topography of G PCL film degraded in col (500 nm × 500 nm), (e) the roughness of non-graft PCL film degraded in col, (f) the roughness diagram of G PCL film degraded in collagenase [Color figure can be viewed at wileyonlinelibrary.com]

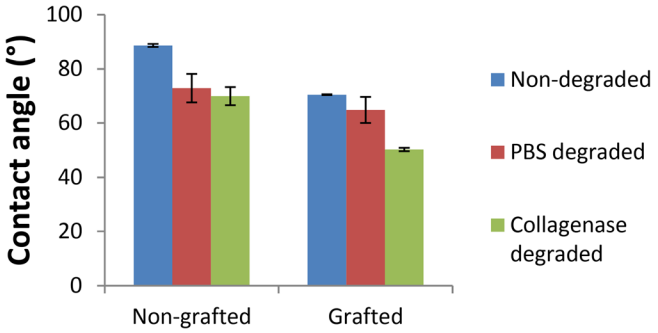

FIGURE 9 Water contact angle of G and NG films after 24 weeks of degradation in PBS and collagenase solution at 37°C [Color figure can be viewed at wileyonlinelibrary.com]

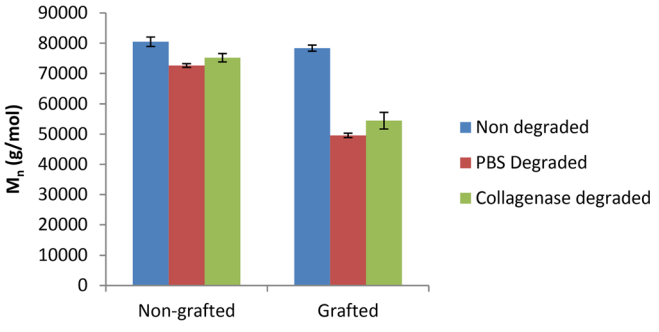

FIGURE 10 Number molecular weight of G and NG samples degraded in PBS and collagenase solution for 24 weeks at 37°C the M_n reduction was statically smaller in samples G in collagenase solution ($p < 0.05$) [Color figure can be viewed at wileyonlinelibrary.com]

erosion mode) endured a reduction of number average molecular weight of 6% against 10% of NG films degraded in PBS (bulk hydrolysis). For G films (erosion and bulk hydrolysis combined), once again, the M_n reduction was more expressive than in NG, however, the decrease was less drastic than in PBS (30% and 36% respectively, Figure 10).

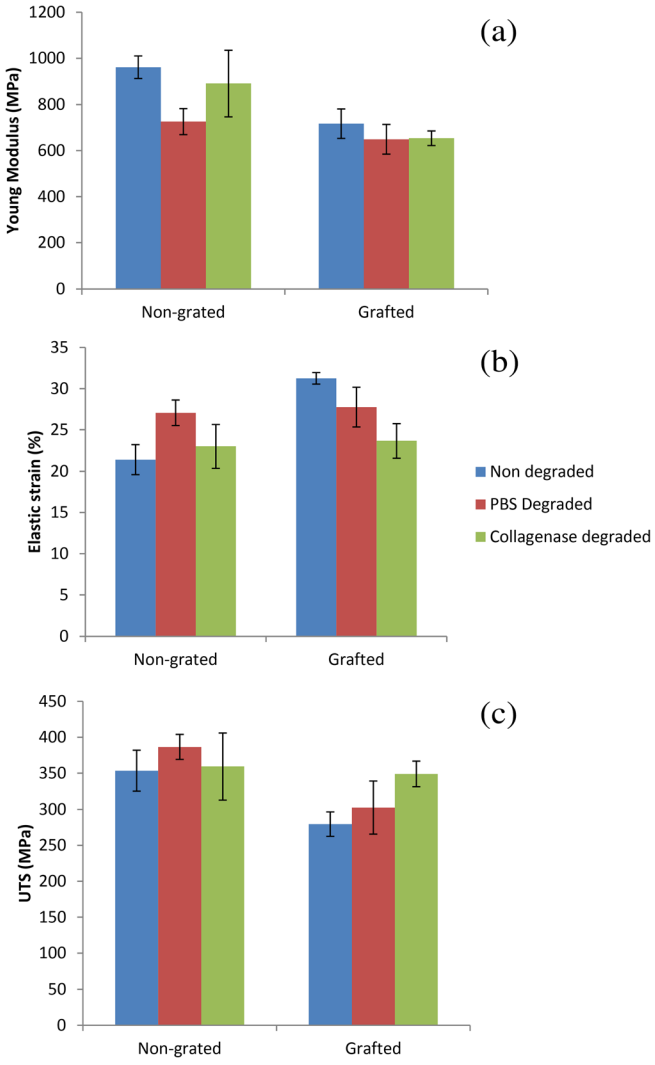

FIGURE 11 Young modulus, elastic deformation and ultimate tensile strength of poly(ε-caprolactone) (PCL) fibers after 24 weeks of degradation in different degradations environment [Color figure can be viewed at wileyonlinelibrary.com]

In the same way, no significant variation in Young Modulus, elastic deformation or ultimate tensile strength was verified for NG samples degraded in collagenase (Figure 11). The values of all these parameters remain almost unchanged even after 6 months of enzymatic degradation, indicating very little modification in the core of the material and supporting again the premise that the degradation was limited to the surface. For the G samples, however, the cracks of hydrolysis were enough present to induce a modification on the mechanical behavior, bringing the values to the same levels of samples degraded in PBS. As previously discussed, the reduction of those parameters should not be a limiting factor for the application since all values were above the values found in the natural ligament.[3,32]

4.3 | Degradation effect on in vitro response

To evaluate the consequences of the degradation on cell behavior, primary sheep ACL cells were cultivated over G and NG medical-grade PCL films after 24 weeks of degradation in PBS and collagenase solution. Cell viability was evaluated after 24 hours of culture by MTT assay. The normalized absorbance indicates the reduction of the tetrazolium salt into formazan crystals by cell mitochondrial activity. The values were compared to the absorbance of non-degraded films. The results can be seen in Figure 12(a).

Similarly to previously studied G surfaces,[35] the results showed higher cell viability (more than 40%) on samples with pNaSS over NG equivalents. Besides, no statistical difference was verified between the viability of the cells cultivated on non-degraded PCL and after 24 weeks of experiment regardless of the degradation medium, and in consequence, the degradation mode. The cell morphology on the surfaces was studied using, as described above, eosin/hematoxylin staining. The analysis of the images of different surfaces—G and NG, degraded or not—at 1, 3 and 7 days of culture (Figure 12 (b)) showed a higher cell spread from D1 and cell density from D3 on the pNaSS G surfaces.

Furthermore, the degradation of PCL in PBS and collagenase does not modify spreading or cell proliferation compared to non-degraded samples, confirming that, in this case, the degradation has no impact on the biological response.

Besides confirming the low cytotoxicity of PCL degradation products,[36] these results were particularly interesting because, as seen in Figure 13, the chemical characterization of degraded PCL surfaces shows a reduction in the grafting density after 24 for weeks of degradation for both PBS and collagenase.

Despite a decrease of about 40 to 50% (bulk hydrolysis and erosion/hydrolysis respectively), the "biological" efficiency of pNaSS was maintained: even when present at low density the pNaSS was capable to modulate the conformation of adsorbed proteins,[37] making it more accessible to cells integrin and improving the cellular response.

5 | CONCLUSIONS

The study of the degradation of PCL showed that the degradation changes the Physico-chemical and mechanical properties of the material and this phenomenon was amplified by the pNaSS grafting procedure. As a matter of fact, the activation of the surface by ozonation makes

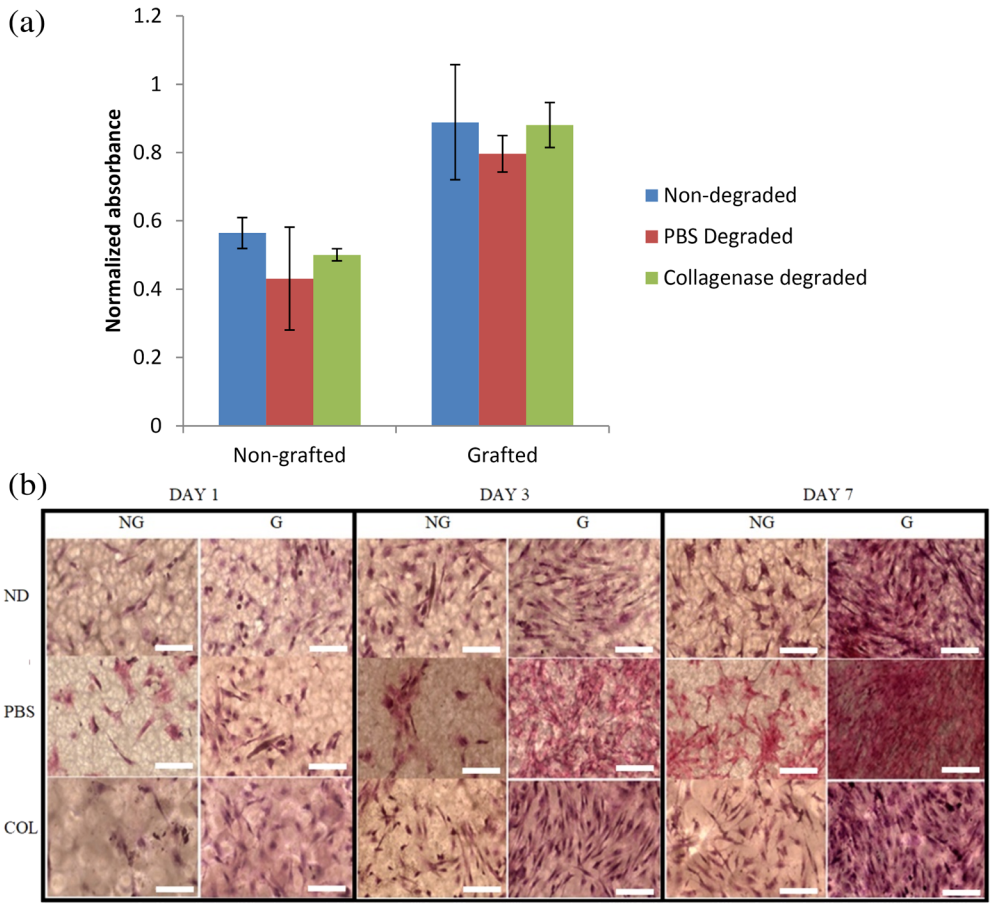

FIGURE 12
(a) Normalized MTT absorbance for G and NG films degraded for 24 weeks in PBS or 50 mg/ml collagenase solution at 37°C; (b) cell morphology (primary sheep fibroblast) after 1, 3 and 7 days of culture on G and NG films before and after 24 weeks of degradation in PBS 50 mg/ml collagenase solution at 37°C [Color figure can be viewed at wileyonlinelibrary.com]

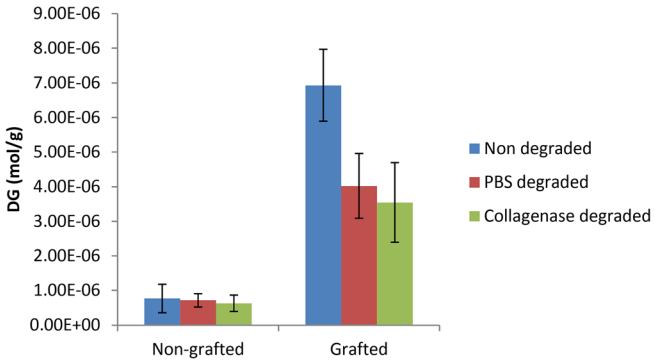

FIGURE 13 Poly(sodium styrene sulfonate) (pNaSS) grafting density over medical-grade poly(ε-caprolactone) (PCL) before and after 6 months of degradation in PBS or 50 mg/ml collagenase solution at 37°C. virgin PCL samples were used as control of non-specific absorption [Color figure can be viewed at wileyonlinelibrary.com]

it more hydrophilic and therefore more sensitive to hydrolysis. This process accelerates the degradation. However, this result is not deleterious for ligament application since after 72 weeks of degradation the medical-grade PCL G with pNaSS has mechanical characteristics superior to those of the natural ligament. Moreover, in parallel with the degradation, the favorable effect of the pNaSS allows the cell colonization over the PCL to strengthen the biodegradable structure with a natural, functional and elastic tissue.

The environment changed the degradation mode and, in the presence of collagenase, the samples were degraded by surface erosion in addition to the bulk hydrolysis for the G PCL. This finding implies that in case of inflammation the surface will be more degraded than in regular conditions, however, in none of the studied cases the degradation was severe enough to decrease the density of pNaSS to levels that could harm its benefic action on cells behavior.

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SUPPORTING INFORMATION

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