Investigation on the properties of linear PLA-poloxamer and star PLA-poloxamine copolymers for temporary biomedical applications.

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A B S T R A C T

The objective of this work was to develop and study new biodegradable thermoplastics with improved mechanical properties for potential use as temporary implantable biomaterials. Linear poloxamer and star-shaped poloxamine have been used as macroinitiators for the ring-opening polymerization (ROP) of lactide to yield high molecular weight PLA-based thermoplastic block copolymers. The influence of the nature of the macroinitiator, PLA crystallinity and initial molecular weight on the copolymers properties was investigated by performing a 7-week degradation test in PBS. The evaluation of water uptakes and molecular weights during the degradation pointed out an early hydrolytic degradation of the 100-kg·mol^{-1} copolymers compared to the 200-kg·mol^{-1} ones (molecular weight decrease of ca. 40% and 20%, respectively). A dramatic loss of tensile mechanical properties was also observed for the 100-kg·mol^{-1} copolymers, whereas the 200-kg·mol^{-1} copolymers showed stable or even slightly improved properties with Young's moduli around 500 MPa and yield strains around 3% to 4%. Finally, the cytocompatibility of the more stable 200 kg·mol^{-1} copolymers was confirmed by murine mesenchymal stem cells (MSCs) culture.

1. Introduction

Synthetic aliphatic polyesters derived from lactide (LA), glycolide (GA), ε-caprolactone (CL) and their copolymers are among the most widely used degradable polymeric biomaterials for medical applications including implantable devices [1], drug delivery systems [2–4] or tissue engineering materials [5–7]. Although particularly popular thanks to its good biocompatibility, poly(lactide) (PLA) does not meet optimal properties to replace soft tissues like ligaments, tendons, cartilage or blood vessels [8–10].

Different strategies are currently used to modify PLA and obtain temporary implantable biomaterials with improved mechanical properties. Cross-linking is one of the most studied techniques [11–15] as it leads to the formation of structures similar to vulcanized rubbers. One of the drawbacks of these materials is that they cannot be remolded by heating or solvent casting because of their cross-linked structure, which makes them difficult to design with complex shapes. Another interesting way is the synthesis of thermoplastic elastomers (TPE), which involves two polymers: a first one is acting as a rigid and hard segment responsible for mechanical strength and a second flexible and soft segment to yield elasticity. Several groups have prepared such materials by associating rigid PLA blocks with more flexible segments like poly(ε-caprolactone) (PCL) [16,17], polyethylene glycol (PEG) [18] or poly(trimethylene carbonate) (PTMC) [19,20], or by chain extension [21,22]. However, it remains difficult to obtain biocompatible and biodegradable thermoplastic materials with high mechanical strength. In particular, most of the above-mentioned polymers exhibit low Young's moduli (in the range 5 to 70 MPa) or low yield stress values (in the range 1–10 MPa) [16,17,20].

The objective of this work is therefore to propose alternative degradable PLA block and star copolymers with improved mechanical properties to be used as temporary implantable materials. Our group being involved in the synthesis and study of PLA-PEG block copolymers for many years [18,23,24], we were interested in evaluating high molecular weight PLA–poloxamer–PLA and PLA–poloxamine–PLA as thermoplastic and biodegradable block copolymers. As far as we know, such copolymers have mainly been used as nanoparticles, microspheres or hydrogels for drug delivery applications and not as materials for biomedical substrates. In association with semi-crystalline PLA, which is biocompatible and biodegradable but has too high mechanical properties when used alone, linear poloxamer and 4-arm poloxamine have been chosen as macroinitiators to confer some elasticity to the material thanks to their structure containing PEG flexible blocks. Besides, four-arm poloxamine should have the ability to generate chain entanglements and physical cross-linking in the polymer network. Associated with different lengths of PLA blocks, these polyether soft segments can change the mechanical properties and the hydrophilic–hydrophobic balance in order to

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modulate the degradation rate. Finally, keeping *in vivo* implantation in mind, we have evaluated the influence of some key parameters like the nature of the central block, the molecular weight and the PLA crystallinity on the materials properties during degradation. A particular attention has been paid to the evolution of molecular weight, thermal properties and mechanical properties. The cytocompatibility of the more promising candidates was finally assessed by studying the viability of murine mesenchymal stem cells (MSCs).

2. Materials and methods

2.1. Materials

Poloxamine (Tetronic® 1107; 15,000 g/mol) was purchased from BASF (Levallois Perret, France). Poloxamer (Pluronic® F-127; 12,600 g/mol), tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, 95%), dichloromethane (DCM), diethyl ether and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St-Quentin Fallavier, France). D-Lactide (D-LA), L-lactide (L-LA) and D,L-lactide (D,L-LA) were purchased from Purac (Lyon, France). PrestoBlue™, Dulbecco's modified Eagle's medium (DMEM/F-12), phosphate-buffered saline (PBS), sterile Dulbecco's phosphate-buffered saline (DBPS), fetal bovine serum (FBS), penicillin, streptomycin and glutamine were purchased from Invitrogen (Cergy Pontoise, France). BD Falcon™ Tissue Culture Polystyrene (TCPS) 24-well plates were purchased from Becton Dickinson (Le Pont de Claix, France) and Viton® O-rings from Radiospares (Beauvais, France). All chemicals and solvents were used without purification.

2.2. Characterizations

The number average molecular weight (M_n) and the polydispersity (PD) of the polymers were determined by size exclusion chromatography (SEC) using a Viscotek GPCMax autosampler system fitted two Viscotek LT5000L mixed medium columns (300 × 7.8 mm), a Viscotek VE 3580 RI detector. The mobile phase was THF at 1 mL/min flow and 30 °C. Typically, the polymer (20 mg) was dissolved in THF (2 mL), and the resulting solution was filtered through a 0.45-μm Millipore filter before injection of 20 μL of filtered solution. M_n was expressed according to calibration using polystyrene standards.

^{1}H NMR spectra were recorded at room temperature using an AMX300 Bruker spectrometer operating at 300 MHz. Deuterated chloroform was used as solvent, chemical shifts were expressed in ppm with respect to tetramethylsilane (TMS). Differential scanning calorimetry (DSC) measurements were carried out under nitrogen on a Perkin Elmer Instrument DSC 6000 Thermal Analyzer. Samples were submitted to a first heating scan to 200 °C followed by a cooling (10 °C.min^{-1} from 200 °C to 100 °C, and 7 °C.min^{-1} from 100 °C to −30 °C) and a second heating scan to 200 °C (10 °C.min^{-1}). Glass transition temperature (T_g) crystallization temperature (T_c), melting temperature (T_m) and melting enthalpy (ΔH_m) were measured on the second heating ramp.

2.3. Copolymers syntheses

PLA–Pluronic–PLA and PLA–Tetronic–PLA block copolymers were synthesized following a procedure previously described by our group [25]. Typically, predetermined amounts of LA and Pluronic or Tetronic were introduced into a flask, and $Sn(Oct)_2$ (0.1 molar % with respect to LA units) was then added. After degassing, the flask was sealed under vacuum, and polymerization was allowed to proceed at 110 °C. After 5 days, the copolymer was recovered by dissolution in DCM and precipitation in cold diethyl ether. Finally, the product was dried under reduced pressure to constant mass. The copolymers were obtained with an average yield of 86% (43 g).

^{1}H NMR: (300 MHz; $CDCl_3$): δ (ppm) = 5.1 (q, 1H, CO-C*H*(CH_3)-O), 3.6 (s, 4H, CH_2-CH_2-O), 3.5 (m, 2H, CH(CH_3)-CH_2-O), 3.4 (m, 1H, C*H*(CH_3)-CH_2-O), 1.5 (m, 3H, CO-CH(CH_3)-O), 1.1 (m, 3H, CH(CH_3)-CH_2-O).

Polymerization degree of PLA blocks and molecular weight of the synthesized triblock copolymers were calculated using the following equations.

$$DP_{PLA} = DP_{PEO}/(EO/LA) \quad (1)$$

For PLA–Pluronic–PLA copolymers:

$$M_n = 2 \times (DP_{PLA} \times 72) + M_{n\ \text{Pluronic}} \quad (2)$$

For PLA–Tetronic–PLA copolymers:

$$M_n = 4 \times (DP_{PLA} \times 72) + M_{n\ \text{Tetronic}} \quad (3)$$

where EO/LA is the ratio of ethylene oxide and lactyl units and PO/LA is the ratio of propylene oxide and lactyl units calculated from ^{1}H NMR spectra.

2.4. Tensile tests

Sample plates were prepared by compression of the polymer in stainless steel mould for 10 min at 200 °C and 8 tons using a Carver press (4120). For tensile mechanical tests, plates (30 × 10 × 0.5 mm) were analyzed at 37 °C in an Instron 4444 at a crosshead speed rate of 5 mm/min and each sample being loaded to failure. Each sample was analyzed in triplicate and Young's modulus (E, MPa), stress at failure (σ_f, MPa), strain at failure (ε_f, %), yield stress (σ_y, MPa) and yield strain (ε_y, %) were expressed as the mean value of the three measurements. E was calculated using the initial linear portion of the stress/strain curves.

2.5. Degradation tests

Sample plates were prepared by compression molding as previously described. Samples were cut (30 × 10 × 0.5 mm), precisely weighed (initial weight W_i) and were then placed in 8 mL of PBS (pH 7.4) at constant temperature (37 °C) under stirring. At scheduled time points, samples were removed from PBS, weighed (hydrated weight W_h), and their tensile mechanical properties were evaluated. After drying to constant mass, they were weighed (dry weight W_d). Water uptake and weight loss of the samples were then calculated from the following equations.

$$\text{Water uptake}(\%) = 100 \times (W_h - W_d)/W_d \quad (4)$$

$$\text{Weight loss}(\%) = 100 \times (W_i - W_d)/W_i \quad (5)$$

Molecular weights and PD were determined by GPC. Molecular weights were also determined by ^{1}H NMR spectroscopy. Thermal properties were measured by DSC.

2.6. Cytocompatibility

Murine mesenchymal stromal cells C3H10T1/2 (designated C3 cells or C3 MSCs thereafter) were used to assess the *in vitro* cytocompatibility of the materials. C3 cells were cultured in proliferative medium composed of DMEM/F-12 without phenol red supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL) and glutamine (2 mM). Sample disks were cut (ø 15 mm) from copolymer films and disinfected in ethanol for 30 min before immersion in a solution of sterile PBS containing penicillin and streptomycin (1 mg/mL) and incubation for 48 h at 37 °C. Disks were then rinsed 2 times with sterile PBS before soaking for 12 h in sterile PBS. After disinfection, disks were placed in TCPS 24-well plates, and non-cytotoxic Viton® O-rings [26,27] were used to maintain the samples on the bottom of the wells and avoid cells growing on TCPS underneath the samples. Disks and empty TCPS

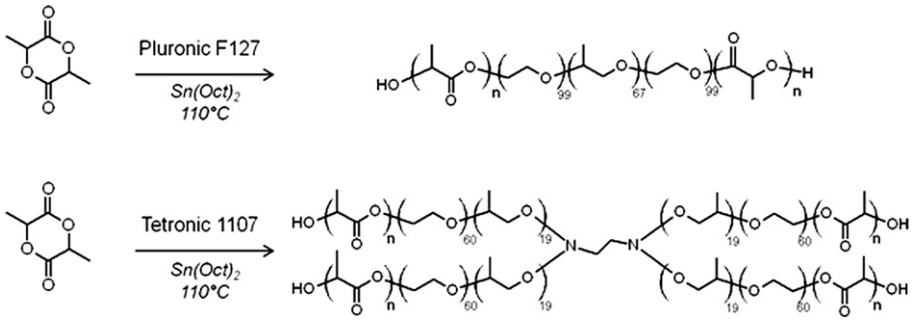

Fig. 1. General synthesis scheme of copolymers.

wells (positive control) were finally seeded with 10^3 C3 cells, which were then maintained in proliferative medium. Viability was evaluated after 2, 4 and 7 days using PrestoBlue assay, a cell-permeable resazurin-based viability reagent that reflects the number of living cells present on a surface at a given time point. At scheduled time points, proliferative medium was removed and replaced by 1 mL of fresh medium containing 10% of PrestoBlue. After 2 h of incubation at 37 °C, 200 μL of supernatant was taken from each well and analyzed for UV absorbance at 570 nm (as the experimental wavelength) and 595 nm (as the normalization wavelength) with a Thermo Scientific Multiscan FC microplate photometer. Values and standard deviations correspond to separate experiments done in triplicate. Homogeneity of variances was checked using Bartlett's test, and one-way ANOVA was applied to determine the statistical significance for multiple comparisons (a level of $p < 0.05$ was considered statistically significant).

Cell adhesion and proliferation were also assessed using PromoKine Live/Dead cell staining kit (PromoCell GmbH, Heidelberg, Germany). After culture in proliferative medium, 10^3 cells were seeded on disinfected polymer disks (ø 15 mm) maintained with O-rings on the bottom of TCPS 24-well plates. After 7 days, proliferative medium was removed and disks were rinsed 3 times with sterile PBS to eliminate non-adherent cells. C3 cells were then incubated in the presence of live/dead staining solution (2 μM Calcein-AM and 1.5 μM propidium iodide) at 37 °C for 15 min and fixed with 3.7% paraformaldehyde for 10 min. Cells were observed under a Leica TCS SP5 X confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany).

3. Results and discussion

3.1. Syntheses

With the aim of providing new degradable copolymers with original mechanical and degradation properties that are compatible with cell culture and tissue reconstruction, we took interest in copolymerizing bioresorbable and biocompatible thermoplastic materials combining PLA segments with Pluronic and Tetronic. One advantage of this strategy is the possibility to modulate the material properties as a function of some parameters controlled by the copolymers design: composition and architecture of the central block, molecular weight, glass transition temperature (T_g), hydrophilic–hydrophobic balance and crystallinity.

In first place, we have evaluated the influence of crystallinity on the copolymers mechanical properties by synthesizing copolymers with PLA_{50}, PLA_{70} and PLA_{94}. This preliminary study (Fig. S1) pointed out as expected that copolymers containing PLA_{94} had much better mechanical properties compared to PLA_{50} and PLA_{70}, especially after immersion in PBS. This observation led us to focus afterwards on copolymers with highly crystalline PLA blocks.

Following this preliminary study, six different copolymers were synthesized (Fig. 1). The influence of some macromolecular parameters on the *in vitro* degradation was studied. These parameters included the nature of the central block (Pluronic F127 or Tetronic 1107), the crystallinity of the PLA blocks, which was controlled thanks to the copolymerization of defined amounts of D-LA and L-LA (PLA_{94} or PLA_{96}), and the global

Table 1
Copolymers properties.

Copolymer	Composition	L-LA	D,L-LA	Initiator	M_n (kg·mol^{-1})		Đ	T_g	T_m	ΔH_m
		(wt%)	(wt%)	(wt%)	NMR	SEC		(°C)	(°C)	J/g
94P100	PLA_{94} Pluronic 100 kg·mol^{-1}	76.9	10.5	12.6	123.3	65.0	1.6	34.4	141.4	12.4
96P100	PLA_{96} Pluronic 100 kg·mol^{-1}	80.4	7.0	12.6	116.4	57.7	1.7	35.4	156.4	16.2
94 T100	PLA_{94} Tetronic 100 kg·mol^{-1}	74.8	10.2	15	115.1	60.3	1.3	34.3	141.8	12.6
94P200	PLA_{94} Pluronic 200 kg·mol^{-1}	82.5	11.2	6.3	223.0	114.0	1.6	53.8	145.4	19.9
96P200	PLA_{96} Pluronic 200 kg·mol^{-1}	86.2	7.5	6.3	222.6	89.0	1.7	58.1	155.6	25.1
94 T200	PLA_{94} Tetronic 200 kg·mol^{-1}	81.4	11.1	7.5	240.4	93.3	1.6	43.9	147.8	10.5

molecular weight of the copolymers (100 kg·mol^{-1} or 200 kg·mol^{-1}). Copolymers properties are summarized in Table 1.

Molecular weights of the copolymers were firstly determined by ^{1}H NMR spectroscopy, thanks to Eqs. (1) to (3). Results were close to theoretical values, although a little higher. On the opposite, SEC analyses gave molecular weight values about two times lower than those calculated by NMR. This difference is explained by the use of polystyrene standards for SEC calibration causing inaccurate measurements with aliphatic polyesters [28,29]. Moreover, the amphiphilic nature of the copolymers can modify their hydrodynamic volume and increase their retention time, giving underestimated results. Molecular weights calculated from ^{1}H NMR spectra are therefore more accurate since only copolymers but no PLA homopolymers were formed during the ROP as confirmed by the monomodal chromatograms obtained by SEC analyses and the spectra obtained by DOSY analyses (Fig. S2). As a consequence, values deduced from ^{1}H NMR analyses can be considered as sound.

DSC analyses of the copolymers mainly showed an increase of T_g and T_m with the crystallinity of PLA blocks and molecular weight. In copolymers containing Pluronic, the molecular weight increase resulted in an increase of ΔH_m and therefore of crystallinity. This was not observed for the Tetronic-based copolymers, because the star structure probably hinders crystallization. Regarding T_g values that are of interest when considering implantation, it is remarkable that increasing the molecular weight of copolymers from 100 to 200 kg·mol^{-1} increased the T_g of about 20 °C from ca. 35 °C to 55 °C. This is of importance since it allows to guaranty minimal mechanical properties changes of the high molecular weight thermoplastics in the eventuality of *in vivo* implantation.

3.2. Water uptake

Water uptake and weight loss of the copolymers were evaluated by weighing samples before and after degradation and were calculated from Eqs. (4) and (5). Results are shown in Fig. 2. Copolymers (200 kg·mol^{-1}) presented low and stable water uptakes during the degradation with values under 10% even after 7 weeks. On the opposite, 100 kg·mol^{-1} copolymers presented a higher absorption (up to 25% for 94T100) in agreement with their higher hydrophilicity resulting from a higher proportion of PEO blocks. As expected, crystallinity also influenced the water uptake, which increased up to 25% for the 100-kg·mol^{-1} samples with PLA_{94} blocks, whereas it remained stable and around 11% in the more crystalline PLA_{96} block copolymers.

3.3. Molecular weights and weight loss

Fig. 3 shows the evolution of M_n (measured by SEC) of the copolymers as a function of the degradation time. M_n decrease was observed for all copolymers with a faster average rate for 100 kg·mol^{-1} copolymers, corresponding to 41% of the initial M_n compared to 22% for

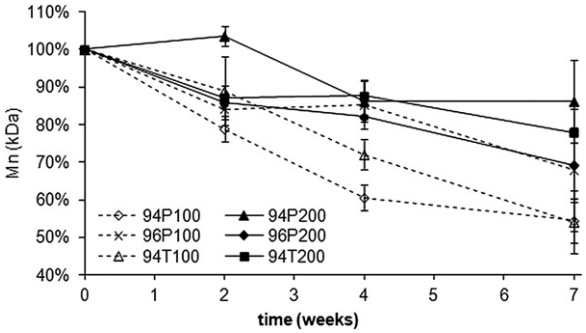

Fig. 3. Molecular weights of copolymers during degradation measured by SEC.

200 kg·mol^{-1} copolymers at 7 weeks. It is to note that molecular weight distribution remained monomodal during the degradation with PD values in the range 1.5 to 2.0, which corresponds to a typical first step of hydrolytic degradation for this kind of PLA-based copolymers [30,31]. The higher degradation rates of the 100-kg·mol^{-1} copolymers compared to the 200-kg·mol^{-1} ones can be attributed to the EO/LA ratio. In agreement with what was observed for the water uptake, the more hydrophilic 100 kg·mol^{-1} copolymers chains soak water more easily, which makes PLA chain hydrolysis faster. In addition, for the 100-kg·mol^{-1} copolymers, it was again found that crystallinity had an influence since the more crystalline 96P100 copolymer degraded more slowly than 94P100 copolymer. On the opposite, no influence of the central Pluronic and Tetronic blocks was found. Finally, regarding the weight loss, they were similar for all samples and limited to a maximum of 5% during the 7-week degradation (data not shown). Again, this is in agreement with the degradation behavior of aliphatic polyesters where weight loss is limited during the early stages of degradation as a consequence of the impossibility for the degraded chains to diffuse out of the samples [23,30,32].

3.4. Thermal properties

Thermal properties were investigated by DSC during the degradation. Thermograms were plotted at various degradation times as exemplified for copolymer 96P200 (Fig. 4). All copolymers showed the same typical evolution. A first noticeable sign of degradation was the appearance of a cold crystallization peak around 120 °C after only 2 weeks. With increasing degradation time, this peak became larger and the cold crystallization temperature (T_c) decreased to about 110 °C. Regarding the melting temperature, a broadening of the melting peak, located in the range 140 to 160 °C depending on the copolymer, was observed as the degradation time increased. This was accompanied by the appearance of a second melting peak 10 °C below the main peak. All these phenomena can be explained by the degradation and shortening of PLA chains. Shorter PLA chains have an increased mobility and are

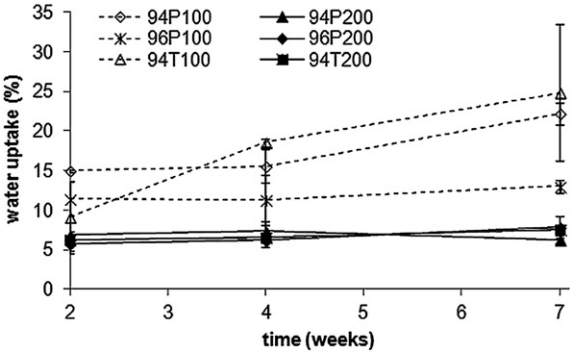

Fig. 2. Water uptake profiles of copolymers during degradation.

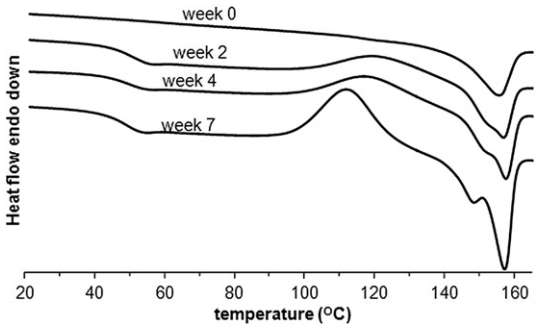

Fig. 4. Thermograms of the copolymer 96P200 during degradation (2nd heating ramp).

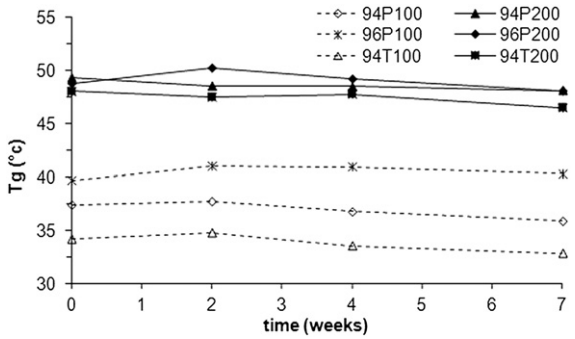

Fig. 5. T_g as a function of degradation time.

thus able to cold crystallize in multiple crystalline structures with different T_m [33,34].

As illustrated in Figs. 4 and 5, degradation did not lead to significant changes of T_g during the 7 weeks of degradation. More interestingly, and in agreement with the initial T_g values already discussed, it is important to note that 200-kg·mol^{-1} copolymers have a higher T_g (in the range 47–50 °C) as a consequence of their high molecular weight, whereas 100 kg·mol^{-1} copolymers have T_g between 30 and 41 °C. Since glass transition may induce major changes in the mechanical behavior of semi-crystalline thermoplastic polymers, it is required for biomedical applications to work with polymers that do not have T_g in the range of physiological body temperature. As a consequence, 100 kg·mol^{-1} copolymers may not be used as implantable biomaterials whereas 200 kg·mol^{-1} copolymers could be further considered for such applications.

3.5. Mechanical properties

Each biological tissue has specific mechanical properties adapted to its function. Consequently, polymers for temporary biomedical applications have to mimic the mechanical behavior of the tissues they are supposed to replace as long as it is necessary, i.e., until tissues repair. For this reason, we were interested in evaluating the main tensile mechanical properties of our copolymers during the degradation process. Fig. 6(a) shows a typical stress–strain curve for our copolymers. This plot can be divided into three distinctive parts: the linear elastic region in which the material holds Hooke's law of elasticity ($\sigma = \varepsilon \cdot E$) followed by, after the yield point, a decrease in the slope which corresponds to irreversible plastic deformation leading to the final damage and failure step. This profile is characteristic of ductile thermoplastic polymers and not of thermoplastic elastomers.

All other graphs in Fig. 6 show the evolution of the main tensile mechanical properties for the 200-kg·mol^{-1} copolymers over the degradation period. Profiles of the 100-kg·mol^{-1} copolymers are not presented here because their faster degradation induced so dramatic losses of their mechanical properties that many of them could not even be placed in the tensiometer grips without breaking. Results observed for the few samples that could have been analyzed were much lower than the 200-kg·mol^{-1} copolymers. Concerning 94P200, 96P200 and 94 T200, all mechanical properties (E, σ_y, ε_y and σ_f) except ε_f remained in the same ranges during

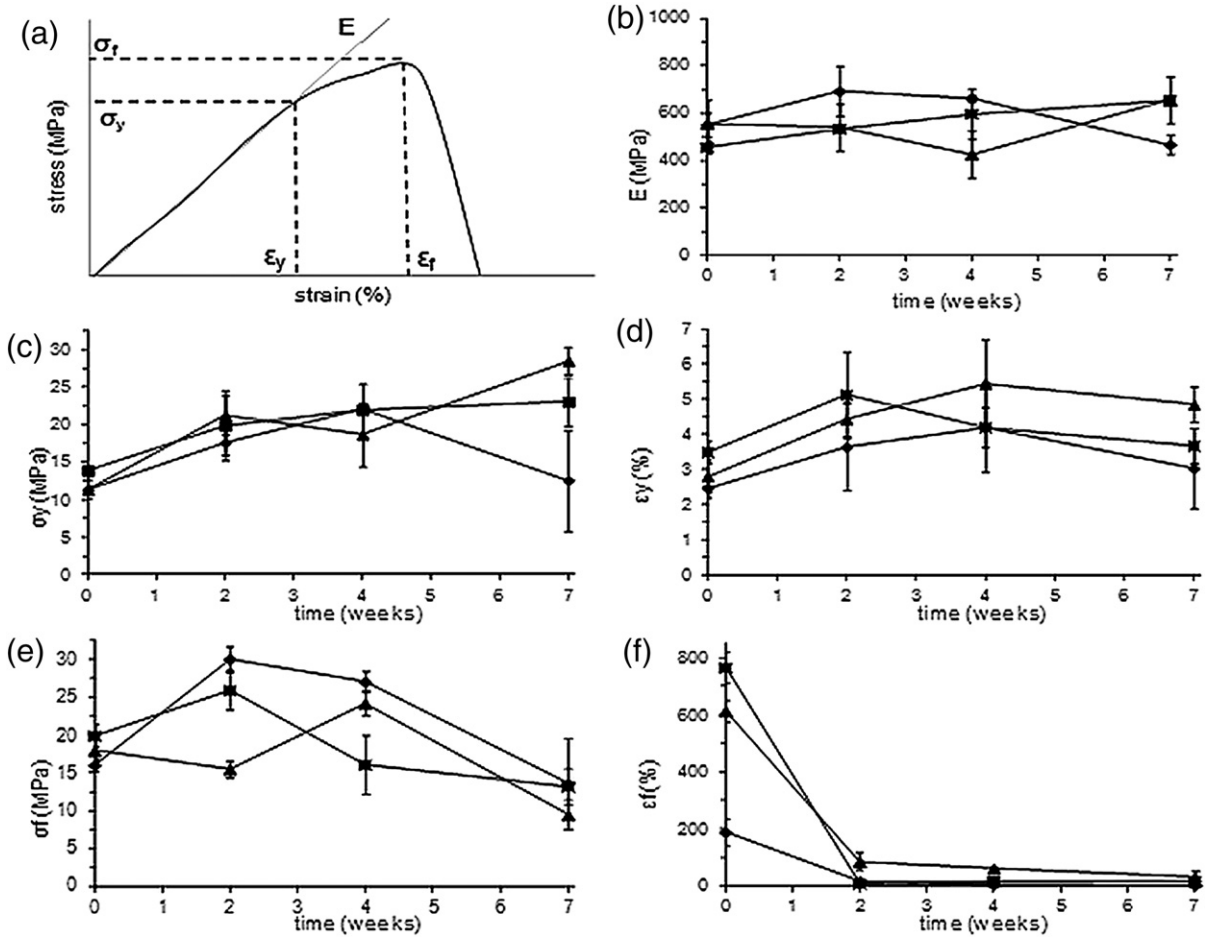

Fig. 6. Evolution of copolymers mechanical properties during degradation. (a) Representation of a typical tensile stress–strain curve obtained for a copolymer. (b) Young's modulus E. (c) yield stress σ_y (d) yield strain ε_y. (e) Stress at failure σ_f. (f) Strain at ε_f failure.

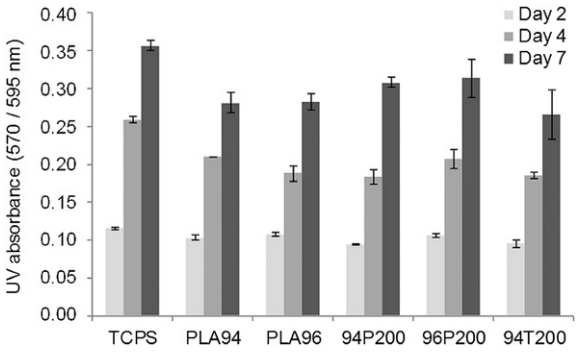

Fig. 7. *In vitro* proliferation of C3 cells on different polymers.

degradation (Fig. 6) with starting values of ca. 10 MPa for σ_y, ca. 2.5–3.5% for ε_y and ca. 20 MPa for σ_f. However, small differences can be found. Indeed, while Young's modulus remained stable in the range 400 to 600 MPa for all copolymers, a common trend seemed to appear for the stress at failure (σ_f), the yield stress (σ_y) and the yield strain (ε_y). In a first step, up to 2 to 4 weeks of degradation, they slightly increased as a result of the initial preferable degradation of the amorphous domains resulting in an increase of crystallinity. This increase is well known for PLA and has been extensively studied by our group in the past [35]. After this initial period, mechanical properties decreased as a result of the polymer chains hydrolysis. In this second period, the decrease of molecular weight (see section 3.3) becomes the main parameter controlling mechanical properties. We can assume that all these properties would steadily decrease until the end of the degradation process.

In opposition to the slow evolution of the other mechanical properties, the strain at failure (ε_f; Fig. 6f) drastically decreased right from the beginning of degradation study for all copolymers that from a ductile behavior turned to a brittle one. This reveals a very strong impact of the chain scission and of the crystallinity increase on this property. It is of importance to note that this decrease corresponded to a drop of ε_f by a factor 20 to 50 depending on the copolymer. In addition, while no difference could be found between the three copolymers in the evolution of the other properties, this is not the case for ε_f. Indeed, the initial strain at failure was higher in the less crystalline 94P200 and 94 T200 copolymers ($\varepsilon_f \approx$ 600–700%) compared to the more crystalline 96P200 copolymer ($\varepsilon_f \approx$ 200%).

3.6. Cytocompatibility

The copolymers have been designed with the FDA approved Pluronic F127 [36] and PLA and the cytocompatible Tetronic 1107 [37]. In addition, to guaranty the overall bioresorbability of the copolymers, the non-degradable central blocks were chosen with molecular weights compatible with renal excretion. To confirm the expected cytocompatibility of the copolymers, the viability and the proliferation of C3 MSCs were tested by seeding the cells at the surface of polymer disks and assessed during a 7-day period. The behavior of the cells on the copolymers was compared to their behavior when cultured on TCPS and 200 kg·mol^{-1} PLA homopolymer (PLA_{94} and PLA_{96}) control samples previously prepared in our laboratory. As shown in Fig. 7, similar level of cell viability was observed for all copolymers with no statistical differences in cell number cultured on copolymers, TCPS or control homopolymers after 7 days. Interestingly, the cells proliferated on the polymer samples with kinetics similar to that observed for cells cultured on TCPS. Cytocompatibility was confirmed with live/dead assay

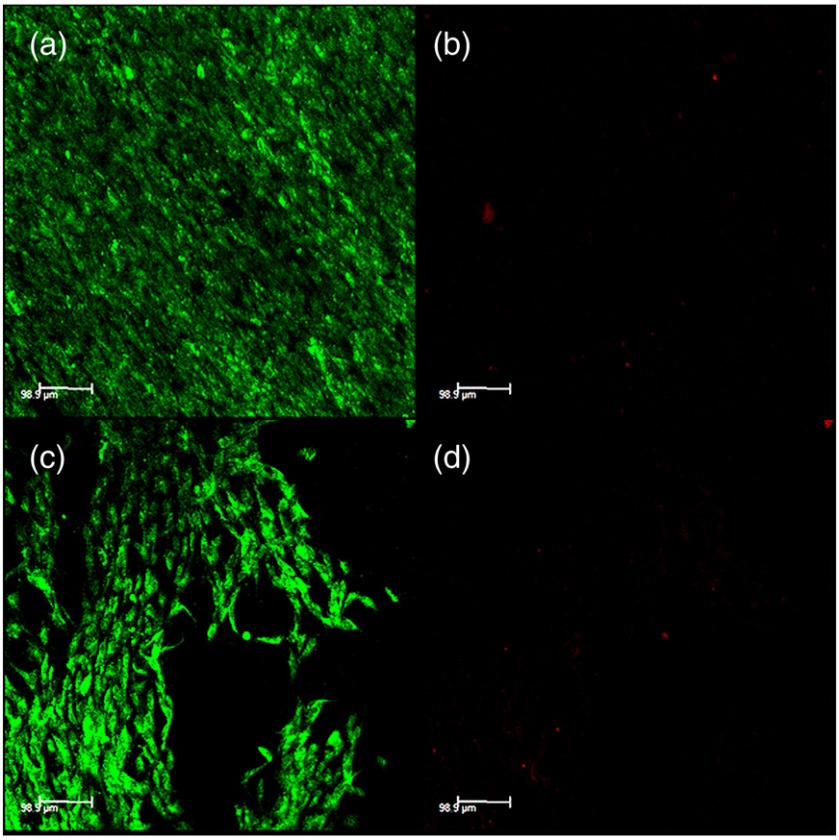

Fig. 8. Live/dead assay fluorescent microscopy pictures after 7 days of proliferation of C3 cells on 94P200 (a,b) and 94 T200 (c, d) copolymers. Staining highlights viable cells in green (a, c) and dead cells in red (b, d).

and confocal microscopy. Fig. 8a shows that C3 cells proliferated and entirely covered the surface of the copolymer. Moreover, almost no red staining was observed in Fig. 8b, indicating no cytotoxic effect on cells. Fig. 8c even shows elongated green C3 cells with a typical fibroblast-like morphology. The results not only confirmed that the copolymers are cytocompatible but also demonstrated that they allow both cell proliferation with normal morphology and high metabolic activity, which will be of benefit for future biomedical applications.

4. Conclusion

In an attempt to develop new degradable biomaterials with specific mechanical properties, and in particular with high Young's moduli, we investigated the synthesis and the short-term degradation properties of PLA-based block copolymers containing Pluronic F127 and Tetronic 1107. This study showed the possibility of modulating the different degradation properties by changing the crystallinity of PLA blocks and the initial global molecular weight. 100 $kg{\cdot}mol^{-1}$ copolymers suffered very important changes during hydrolytic degradation, illustrated by an early water uptake, a rapid molecular weight decrease and a total loss of mechanical properties after 7 weeks. These results, as well as DSC thermograms showing T_g close to the human body temperature, make them inappropriate for biomedical applications when mechanical stability is needed. On the opposite, even though 200 $kg{\cdot}mol^{-1}$ copolymers showed a significant molecular weight decrease during degradation, they kept a higher mechanical integrity with maintained Young's moduli in the range 400 to 600 MPa. Due to their higher crystallinity, the copolymers based on PLA_{96} also exhibited slightly lower strains at failure than those containing PLA_{94}. Degradation rates associated with good cytocompatibility make the 200-$kg{\cdot}mol^{-1}$ star and linear PLA copolymers interesting as materials for temporary biomedical applications requiring cell culture.

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