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Biodegradable polyester networks including hydrophilic groups favor BMSCs differentiation and can be eroded by macrophage action

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ABSTRACT

The aim of this study is to show that introducing a small fraction of hydrophilic groups into a hydrophobic polyester favor the macrophage activity by accelerating the degradation action in aqueous media. It is also seen that differentiation of MSCs cultured in monolayer towards bone in specific differentiation media is favored in these materials with respect to the corresponding pristine polyesters. Polymer networks based in polycarpolactone or poly(L-lactide) and containing a small fraction of poly(-hydroxyethyl acrylate) have been synthesized. Degradation kinetics *in vitro* was monitored by mass loss and swelling capacity of the polymer network in good solvents, the later as representative of chain cleavage. Hydrolytic and enzymatic degradation is accelerated by the inclusion of poly(hydroxyethyl acrylate) blocks in the network. Macrophages were cultured on the surface of the network films, showing its capacity to erode the material surface but also to accelerate bulk degradation. Bone marrow mesenchymal stem cells were cultured in monolayer on the membranes in osteogenic media, showing an increase of specific markers expression in comparison to pristine polyesters.

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1. Introduction

Tissue engineering is defined as an interdisciplinary science that use different materials (such as polymers) to design scaffolds to improve damaged tissue function [1]. Recent tissue engineering techniques use different materials with synthetic or natural origin for the fabrication of scaffolds and hydrogels, with or without previously seeded cells [2–5]. The scaffold and the polymeric materials should besides being biocompatible also resemble the native tissue and guide cell ingrowth and have a controlled degradation rate. Biodegradable polyesters have found many medical applications, from suture thread to screws or plates in traumatology or, as macroporous scaffolds in tissue engineering applications. In all these applications the control of degradation kinetics is important. Both polycaprolactone (PCL) and poly(L-lactide) (PLLA) are hydrophobous semicrystalline polymers. The ester groups of the main chain are susceptible to hydrolysis and thus PCL and PLLA degrades in the presence of water, phosphate-buffered saline (PBS) or lipoprotein lipase [6,7]. Other hydrolytic enzymes do not show significant effect on the degradation rate of these polymers [7–9]. The molecular weight of poly (α-hydroxy acids) decreases fast, in days for poly glycolic acid or weeks in PLLA [10] or months in PCL [11] in aqueous media. Despite this, these materials do not show any mass loss until the low-molecular weight fractions resulting from the cleavage of the polymer chains, are delivered to the surroundings [3].

The degradation rate of polymers is highly dependent of the effective surface to volume ratio, being faster in porous sponges than in thin non-porous films and faster in the later than in bulk samples [6]. It is problematic to compare the *in vitro* and *in vivo* enzymatic degradation due to the difficulty to reproduce the complex environment around the implant in the laboratory. In fact, when the *in vivo* and *in vitro* degradation coincide, this could be considered a sign of hydrolytic degradation [12]. Particularly, the eroding capacity of macrophages through the secretion of a variety of hydrolytic enzymes and reactive oxygen species is crucial in

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some biomaterials [13–15]. Foreign body giant cells have an extended ability to degrade the implanted biomaterial. The adhesion of monocytes, differentiation to macrophages and fusion to form foreign body giant cell is influenced by the surface chemistry and topography of the implanted material [13]. Therefore, the design and composition of the biomaterial is important for biocompatibility and tissue integration.

It is hypothesized that by introducing a hydrophilic component into the structure of a hydrophobic polymer susceptible to hydrolytic degradation, the water diffusion will increase. This will further modify the degradation process towards a bulk cleavage of the polymer chains. Therefore, a series of bulk copolymer networks combining PCL or PLLA blocks with a small amount of poly(-hydroxyethyl acylate) were synthesized following the protocols described in previous works [16–20]. The goal of this study is to assess the degradation of these materials in different media that can be representative of the environment of the materials once implanted in the host tissue. In this way we will demonstrate that the degradation of the networks containing hydrophilic units is much faster than in the corresponding PCL or PLLA homopolymers. On the other hand we aim at probing that hydrophilic units play a central role in inducing the susceptibility of these materials to macrophage erosion. Besides this the osteogenic differentiation of bone marrow Mesenchymal Stem Cells, MSCs, cultured on these materials is studied.

2. Materials and methods

2.1. Polymer synthesis and characterization

PCL and PLLA homopolymer films were prepared as we have previously described [21]. Briefly, PCL and PLLA were dissolved in chloroform and casted into a glass Petri dish. The solvent was allowed to evaporate at room temperature and then the resulting films were dried under vacuum.

PCL-PHEA and PLLA-PHEA copolymer networks containing a 30% by weight of PHEA blocks were synthesized according to previous studies [16,18]. The network hydrogels synthesis consists of three main steps:

2.1.1. Synthesis of poly(L-lactide)diol (PLLA)

L-Lactide (0.15 mol) (Sigma Aldrich, Spain), Tin (II) 2-ethylhexanoate (1.1 mmol) (Sigma Aldrich, Spain) and Ethylene glycol (7.5 mmol) (Scharlab, Spain) were mixed in a three-neck round-bottom flask (at 60 °C for 45 min), under nitrogen atmosphere. Then the pressure was decreased to 430 mbar and the reaction continued with agitation for 5 h. A white precipitate was formed. The polymer was isolated and purified by acetone (99.6%, Sigma Aldrich, Spain) and precipitated on ethanol (99%, Scharlab, Spain). The polymer was characterized by ^{1}H NMR and FTIR. The molecular weight was measured by size exclusion chromatography.

2.1.2. Synthesis of polylactic macromer (mPLLA) and polycaprolactone macromer (mPCL)

The hydroxyl terminal groups of the diol were derivatized with methacrylic anhydride. The synthesis was carried out with PLLA diol (Mw = 8500 g/mol; 0.001 mol) or PCL diol (2000 g/mol; 0.001 mol, Sigma Aldrich, Spain) respectively; the sample was dissolved into a three-neck round-bottom flask with 50 ml of ethyl acetate anhydrous (Scharlab, Spain) on an ice bath. Methacrylic anhydride (25 ml; 0.29 mol, Sigma Aldrich, Spain) was added drop wise, and the reaction was stirred for one hour under nitrogen atmosphere. Then the reaction was stirred for 7 h at 80 °C. The mixture was poured into 500 ml of cold ethanol, centrifuged and dried. The products obtained were mPCL and mPLLA respectively.

2.1.3. Networks hydrogels synthesis of PCL-HEA and PLLA-HEA with a rate of 70/30

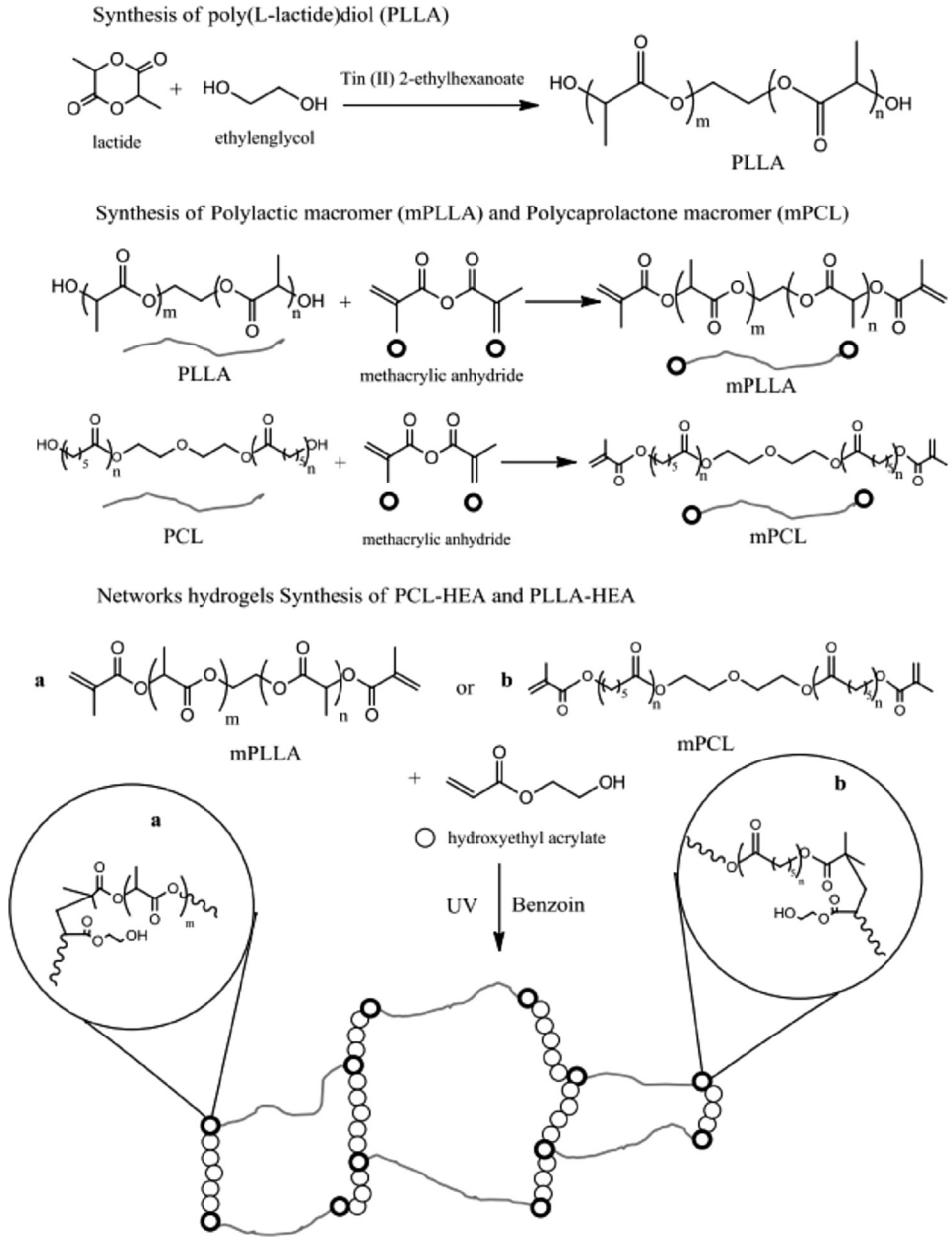
The networks hydrogels were prepared by dissolving mPLLA or mPCL in dioxane (35% w/v, Sigma Aldrich, Spain) and mixed with benzoin and HEA monomer (photoinitiator, 1 wt% of HEA,Scharlab, Spain) respectively. The polymerization was carried out under UV light for 24 h. Low-molar-mass substances were extracted by boiling in ethanol for 24 h and then drying in vacuum until constant weight (Scheme 1).

2.1.4. Materials characterization

Differential scanning calorimetry (DSC) analysis was carried out with a Perkin-Elmer DSC 8000 instrument under a flowing nitrogen atmosphere between 0 and 200 °C at a heating rate of 20 °C min^{-1} for cooling and heating. All samples were measured in 30 μL aluminum pans with perforated lids to allow the release and removal of volatiles. The contact angle of deionized water on the different samples was measured with the equipment "Dataphysics OCA 20" delivered by Dataphysics GmBH-Neurtek S.A. Images were taken always 30 s after the placement of the droplet on the sample.

2.2. Cell culture

Bone marrow stromal cells (BMSCs) and Murine macrophage RAW 264.7 cells were grown in DMEM (Invitrogen, Buenos Aires, Argentina) containing 10% FBS (Natacor, Argentina), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 atmosphere [21]. Bone marrow stromal cells were obtained from rats as described previously and characterized by their ability to differentiate to various phenotypes, such as osteoblasts, adipocytes and chondrocytes. The use of animals to obtain the BMSCs was carried out in conformity with the Guidelines on Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare(1992) [22,23]. Approval for animal studies was obtained from the institutional accreditation committee (FCE-UNLP's Animal Welfare Assurance Nº 019-00-15). Briefly, animals were sacrificed under anesthesia by rapid neck dislocation. BMSCs were collected by flushing the dissected femoral and tibial diaphysis medullary canal with DMEM under sterile conditions. The resulting suspension was seeded in a 25 cm^2 tissue culture flask. Cells were grown in DMEM supplemented with 5% (v/v) FBS, 100 U/ml penicillin and 100 g/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2. For the experiments, polymeric matrixes (films or hydrogels) were cut and placed in a 24-well plate. BMSCs were plated on each matrixes at a density of 5×10^4 cells/well, and cultured in 10% FBS–DMEM at 37 °C. After reaching confluence, the cells were induced to differentiate into osteoblasts using an osteogenic medium: 10% FBS–DMEM containing 25 mg/ml ascorbic acid (Sigma Aldrich, USA) and 5 mM sodium β–glycerol-phosphate (Sigma Aldrich, USA). The media was changed twice a week, and osteoblastic differentiation was evaluated after 15 days by measuring alkaline phosphatase activity (ALP) and type 1 Collagen production. The nodule mineralization deposition after 21 days was assessed by Alizarin Red staining. To determine ALP, cell monolayers were washed with PBS and the total cell extract was obtained with 200 μL 0.1% Triton-X100 (Sigma Aldrich, USA). A 100 μL aliquot of the extract was used to evaluate ALP by hydrolysis of *p*-nitrophenylphosphate (*p*-NPP) (Sigma Aldrich, USA) into *p*-nitrophenol (p-NP) at 37 °C for 1 h. The absorbance of p-NP was recorded at 405 nm [21]. Aliquots of each cell extract were used for protein determination by Bradford's technique [24]. For type I collagen production, cells were fixed with Bouin's solution and stained with Sirius red dye for 1 h. The stained material was dissolved in 1 ml 0.1 N sodium hydroxide and the absorbance of the solution was recorded at 550 nm [25]. To



Scheme 1. Chemical reactions to synthesis the copolymers and networks hidrogeles.

determinate mineral production of BMSCs grown on matrixes, the cells were fixed with formalin 10% in PBS and stained with Alizarin Red (2% in water, pH = 4.2) during 10 min. After removing the dye, the arrays were washed and the dye with 0.1 N NaOH on this extract was solubilized. Protein quantification was performed using spectrophotometry at a wavelength of 548 nm.

2.3. Degradation studies

Degradation was evaluated after 42 days incubation in PBS and PBS with lipase enzyme (25 IU/ml). Degradation was also evaluated after 14 days in DMEM supplemented with 1% FBS with or without RAW264.7 macrophages culture. All experiments were carried out at 37 °C in a humidified atmosphere containing 5% CO_2. Briefly, PCL and PLLA films and PCL-PHEA and PLLA-PHEA hydrogels were cut and weighed (W_0) and incubated at the different conditions during different times. After each time-point, the sample were washed exhaustively with distilled water and for the samples with RAW264.7, the cells were lysed with 0.1% triton X100 and then washed several times with distilled water. After that, samples were dried under vacuum and weighed (W_t). The samples were placed in acetone for 24 h to determine equilibrium acetone absorption. Then, samples were removed and weighed (Wh_{ac}). After that, samples were dried under vacuum and placed in distilled water for 24 to determine equilibrium water absorption; and then removed and weighed again (Wh_{aq}). Degradation was evaluated by calculating the percentage weight loss (equation (1)) and percent swelling in both water (equation (2a)) and acetone (equation (2b)).

$$\%W = \frac{W0 - Wt}{W0} x100 \quad (1)$$

$$\%Swell\ in\ water = \frac{W\ haq - W0}{W0} x100 \quad (2a)$$

$$\%Swell\ in\ acetone = \frac{W\ hac - W0}{W0} x100 \quad (2b)$$

2.4. Topographic changes

Possible changes in the topography of films and hydrogels as a consequence of different degradation treatments were evaluated using scanning electron microscopy (SEM) (Phillips 505, The Netherlands), with an accelerating voltage of 20 kV. The images were analyzed by Soft Imaging System ADDAII. To observe the macrophages RAW264.7 after culture over the supports, samples were washed with PBS, fixed in methanol and dehydrated with ethanol 70°, 96° and absolute ethanol.

2.5. Statistical analysis

Student's T-test was used for comparisons between control and experimental groups. All results are expressed as mean ± standard differentiation and represent at least three different experiments performed in triplicate.

3. Results and discussion

PCL and PLLA (Scheme 1) may degrade by hydrolytic cleavage of their ester groups in the chain backbone [10,11]. Nevertheless, the hydrolytic degradation rates are quite different from each other. The higher number of ester groups in the main chain of PLLA makes it more susceptible to hydrolytic degradation than PCL. In both cases the hydrophobicity of the homopolymers hinders the access of water molecules to the sample core. Crystallinity is another factor hindering the diffusion of water towards the polymer chains, thus degradation is faster in the amorphous phase than in the crystallines. In the case of PCL, the slow degradation kinetic limits its use in some tissue engineering applications. Co-polymerization is a usual strategy to decrease the crystallinity since the regularity required for crystalline order is disrupted by the presence of different monomeric units randomly distributed along the polymer chain. If the co-monomer is hydrophilic, as in the case with hydroxyethyl acrylate, the water absorption increases, facilitating water access to the polymer chains [16,20,26,27].

The block co-polymer networks in this study consists of either PLLA or PCL blocks whose terminal ends are bonded to PHEA chains. In both cases the water contact angle of the networks decrease with respect to that of the corresponding polyester homopolymers, as shown in Fig. 1A, demonstrating a significant increase of hydrophilicity with the introduction of PHEA blocks in the material. Thermal behavior of PCL and PLLA are quite different, while PLLA crystallizes quite slowly, and its glass transition is above room temperature, PCL crystallizes faster, its glass transition temperature is around −60 °C, thus PCL crystallizes at room temperature [28]. These differences made that the influence of the presence of PHEA in the network be different in PCL-PHEA and PLLA-PHEA copolymer networks. In Ref. [16] it was shown that PCL blocks in PCL-PHEA copolymer networks keep a certain capacity to crystallize as shown by DSC studies. Nevertheless, the behavior of PLLA-PHEA system is not the same. Cooling and heating DSC thermograms of PLLA diol show the characteristic behavior of PLLA, that due to its slow crystallization kinetics is not able to crystallize on cooling at 20ºC/min, showing only the glass transition which is quite low due to the low molecular weight of the polymer. On heating, after glass transition crystallization is shown by the exothermal peak followed by melting (Fig. 1B). Interestingly enough PLLA-PHEA samples only shows a single glass transition with no sign of crystallization or melting, probing that crystallization of the polyester chains is impeded by the fixation of the block ends and the miscibility with PHEA blocks. These results agree with previous observations [29].

The decrease of average molecular weight with time is normally seen to be faster than mass loss during the degradation of a polymer [30]. The cleavage of polymer chains start decreasing molecular weight and widening the molecular weight distribution, while depolymerization with mass loss starts later. In the case of a polymer network, the molecular weight is not defined, but the cleavage of polymer chains can be monitored by the increase of solvent uptake capacity.

The Flory-Rehner equation gives [31,32] the relationship between swelling capacity of a polymer network and the number of effective polymer chains between crosslinks per unit volume of polymer, n_c/V(Equation (3)).

$$\ln\left(1 - \phi_{pol}\right) + \phi_{pol} + \chi\phi_{pol}^2 + \nu_{sol}\frac{n_c}{V}\phi_{pol}^{1/3} \quad (3)$$

where ϕ_{pol} is the volume fraction of polymer in the swollen network, v_{sol} is the molar volume of the solvent and χ is the Flory interaction parameter between polymer and solvent. Cleavage of PCL or PLLA chains during hydrolytic or enzymatic degradation decreases the number of effective chains between cross-links, and increase the swelling capacity according to Equation (3).

Basically any good solvent of the polymer network would be suitable for the swelling characterization. For instance, solvent uptake in our original PCL-PHEA block copolymer network, was measured on dry basis, and was 1008% for chloroform absorption, 533% for dioxane, 242% for acetone, 62% for ethanol and 26% for water. Acetone and water were chosen for our study since they permit to get swelling results with enough accuracy.

Acetone and water are quite different to each other with respect to the swelling behavior in our copolymer networks: While acetone is a good solvent for either PLLA, PCL or PHEA, water is a good solvent only for PHEA. As a consequence, it is expected that the equilibrium solvent content will reflect differently the effect of PLLA or PCL chains cleavage due to hydrolytic degradation. Interestingly enough, water absorption of pristine PCL or PLLA was negligible (results not shown). However, when HEA was incorporated in the PLLA-PHEA network the equilibrium water content increased to up to 155% (Fig. 2B), while PCL-PHEA with the same HEA content still absorbed no water (Fig. 2A). The main difference between these two systems is the degree of phase separation between the hydrophilic and the hydrophobic components. In PLLA-PHEA networks the crystallinity of PLLA is completely suppressed by the presence of PHEA chains. Nonetheless two glass transitions are detected by calorimetry, they do approach to each other in the network with respect to that of the homopolymers, indicating a certain miscibility [18]. Nevertheless, phase separation in the PCL-PHEA system permits the formation of PCL crystals and thus, the hydrophilic component is probably segregated in higher extent [16]. This observation shows the importance of the spatial distribution of hydrophilic groups in the network that either allows, or not, water migration to the sample. Still, the PLLA-PHEA network swelled more in acetone (366 ± 4%) than PCL-PHEA (200 ± 6%) before degradation, which also could be influenced by the PCL crystallinity. Fig. 2 shows % of swelling in acetone and in water of the PCL-PHEA (Fig. 2A) and PLLA-PHEA samples (Fig. 2B) after degradation with lipase and PBS. It can be seen that purely

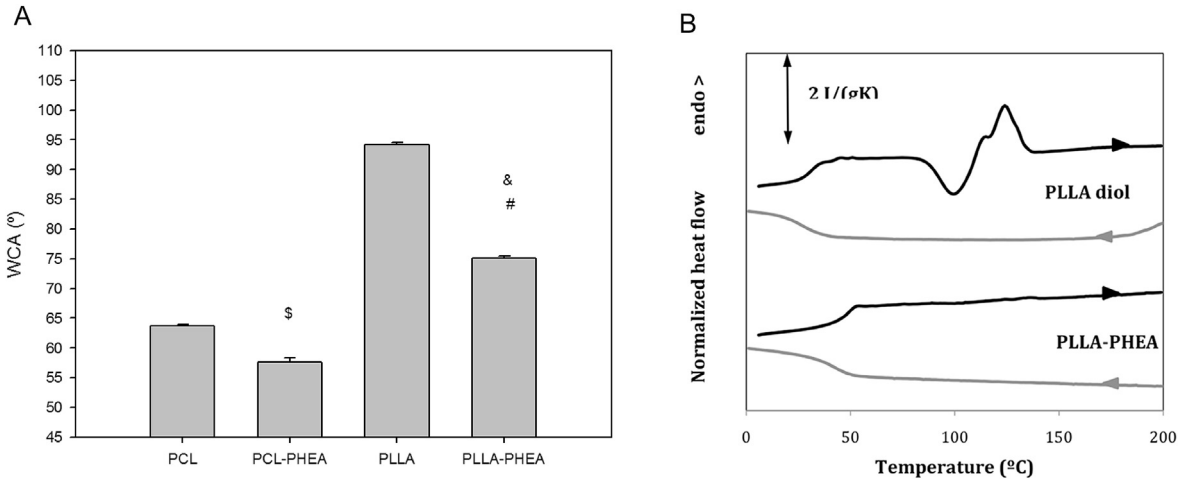


Fig. 1. A. Water contact angle of PCL-PHEA and PLLA-PHEA networks. $: $p < 0.01$ respect to PCL; &: $p < 0.01$ respect to PLA; #: $p < 0.01$ respect to PCL-PHEA. B. DSC cooling and heating thermograms of PLLA diol and PLLA-PHEA systems.

hydrolytic degradation of either PLLA or PCL was very small in PBS as detected by weight loss. Even so, the enzymatic degradation in lipase solution was significant. The introduction of HEA in the systems accelerates the degradation in both media. Fig. 2 shows the % weight loss of the pure PCL film and PCL-PHEA hydrogel (Fig. 2C) and pure PLLA film and PLLA-PHEA hydrogel (Fig. 2D). For example when degraded with lipase, PLLA-PHEA has lost 30 ± 1% of weight while pristine PLLA has lost 6.7 ± 0.4% of weight after 42 days. A similar behavior occurs in the system based in PCL, but the effect of the presence of HEA is not so consequential. The PCL-PHEA network creased 8.5 ± 1.9% in weight, while the PCL only 3.2 ± 1.2%. The effect of HEA segments in the degradation in PBS is analogous but much less pronounced.

These phenomena can be correlated with the increase of

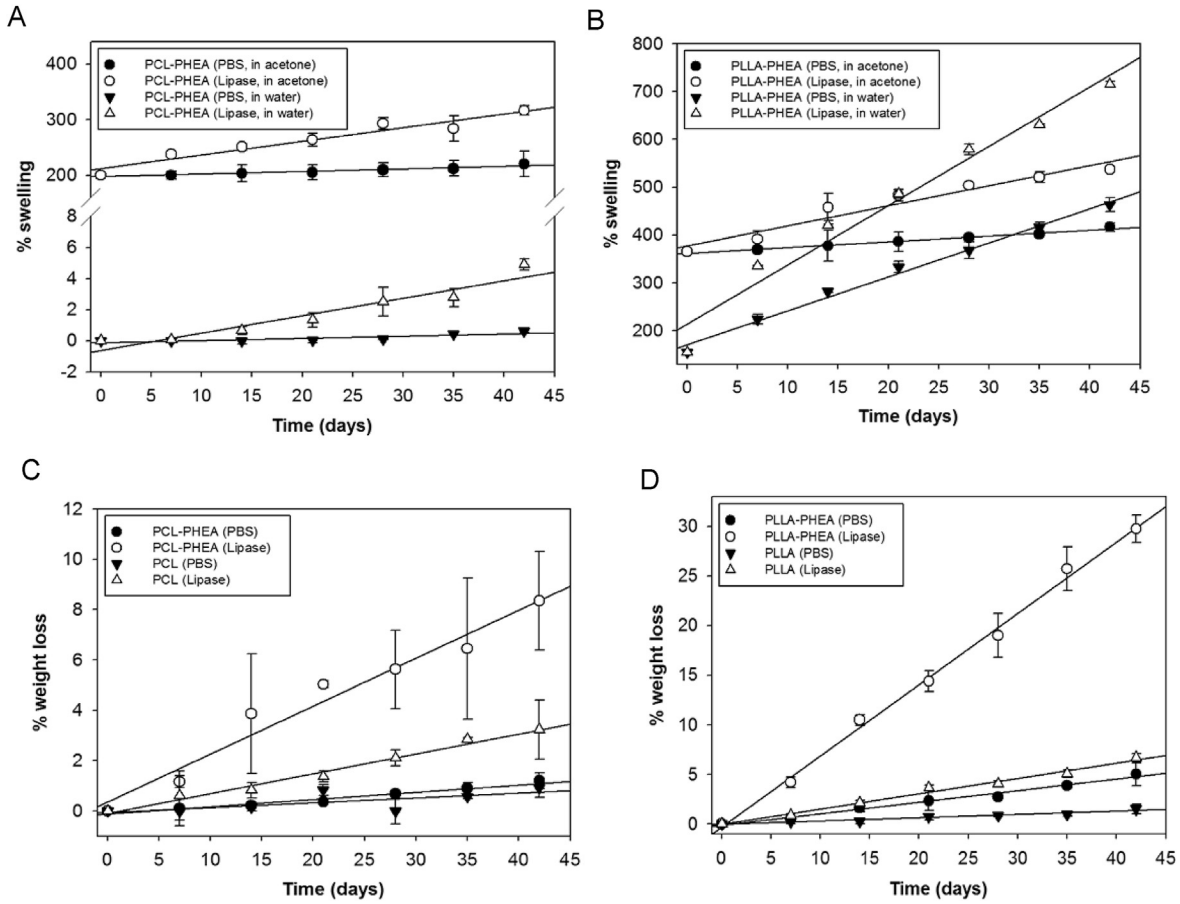


Fig. 2. PLLA and PCL films and PLLA-PHEA and PCL and PHEA hydrogels degraded with lipase or PBS. A. % Swelling of PCL-PHEA in Acetone and water. B. % Swelling of PLLA-PHEA in Acetone and water. C. % weight loss of PCL-PHEA and PCL. D. % weight loss of PLLA-PHEA and PLLA.

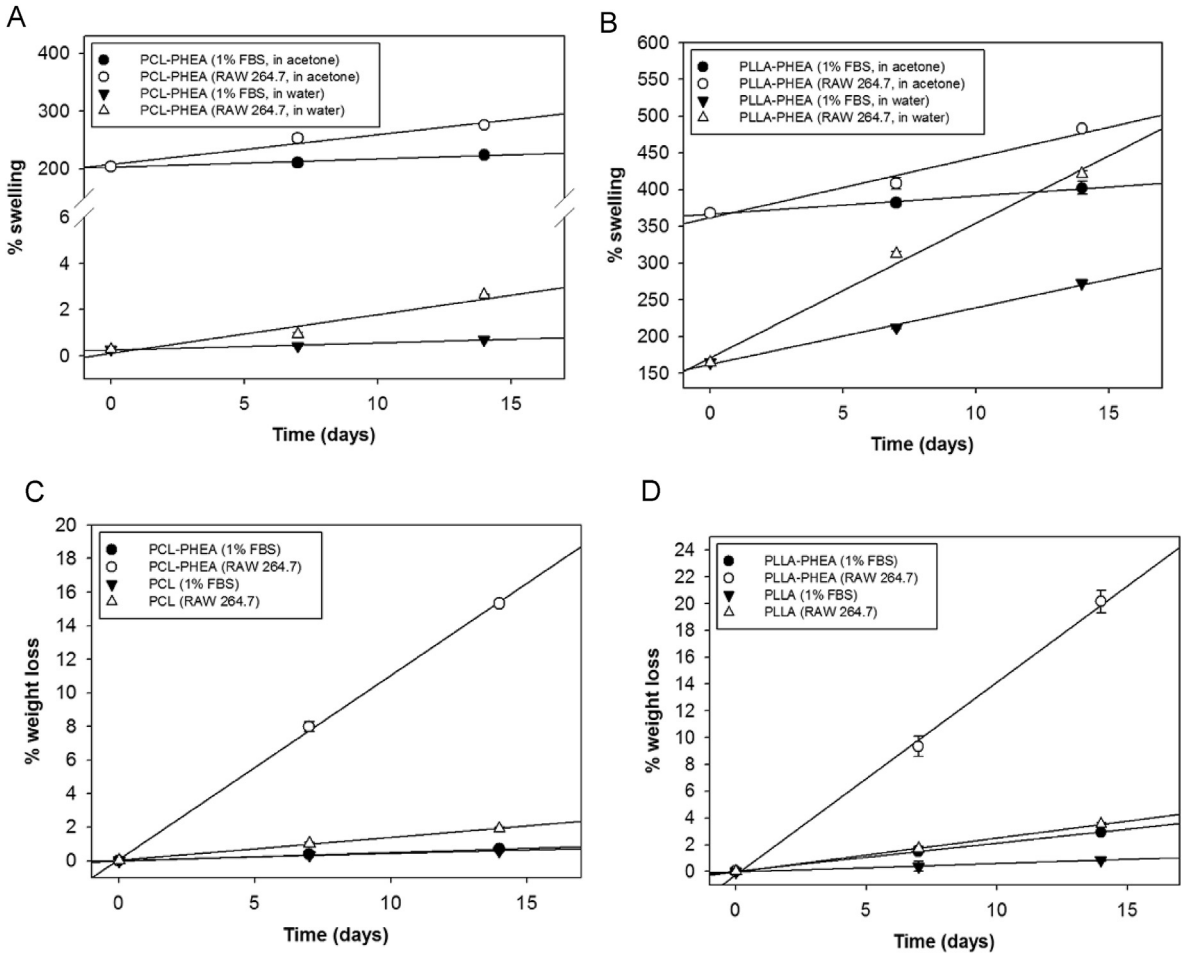


Fig. 3. PLLA and PCL films and PLLA-PHEA and PCL and PHEA hydrogels degraded with RAW 264.7 or 1% SBF in DMEM. A. % Swelling of PCL-PHEA in Acetone and water. B. % Swelling of PLLA-PHEA in Acetone and water. C. % weight loss of PCL-PHEA and PCL. D. % weight loss of PLLA-PHEA and PLLA.

swelling capacity, i.e with the cleavage of polyester chains of the network, since PHEA chains can be completely stable. After 42 days in PBS, PLLA-PHEA absorbed 418 ± 9% acetone, i.e. a 114% of the swelling capacity before degradation (Fig. 1B). In lipase solution PLLA-PHEA absorbed 538% ± 5% acetone (a 37% more than before degradation). Surprisingly enough this network absorbed 463 ± 15% or 715 ± 6% water after degradation in PBS and lipase respectively. The sample absorbs three times more water after degradation in PBS than before degradation, and more than four times after degradation in lipase than before degradation. Cleavage of polylactide chains during degradation increases the swelling capacity of both PLLA and PHEA in acetone, as expected for a good solvent. This is due to the decrease of elastic free energy of the network as the number of effective cross-linking points decrease with the chain cleavage between cross-links. The effect should respond to the Flory-Rehner equation (3). Nevertheless, the fraction of binding points for water in the polymer network is scarce, only 10% fraction of HEA units, and water migration through the PLLA rich medium is hindered. In this case, PLLA chain cleavage has a multiplier effect on water absorption, since it allows enhanced conformational mobility that increases the water diffusion.

The behavior of the PCL-PHEA is quite different (Fig. 2A and 2C). It can be seen that after 42 days in PBS the swelling was 220 ± 23% in acetone which is nearly the same as before degradation, and 0.63 ± 0.12% in water. After degradation in lipase, the acetone absorption was 316 ± 8% (158% of the amount before degradation) and the water absorption was 4.9 ± 0.4%. In this case, degradation is clearly slower than in the PLLA-PHEA and the difference between acetone and water absorption is not so clear. Both factors can be ascribed to a more pronounced phase separation between PCL and PHEA nano domains in the network. It can also be explained by the presence of PCL crystals whose degradation is slower than that of the amorphous phase [11] while PLLA blocks are amorphous in the copolymer network.

Once implanted in a host tissue, biomaterials could be degraded not only by enzymatic mechanisms but also under the action of macrophages. Fig. 3 shows % swelling and % weight loss of the films and hydrogels after up to 14 days of degradation by RAW 264.7 cells in DMEM +1% FBS, and only DMEM +1% FBS without RAW 264.7 as control. The weight loss in presence of macrophages nearly doubles that found in lipase solution for the same degradation time both in PLLA-PHEA (Fig. 3D) and PCL-PHEA (Fig. 3C) networks. Fig. 3C and D shows the huge difference in the degradation rate between the samples containing or not PHEA segments in the macrophage culture. Clearly the introduction of PHEA segments makes both PLLA-PHEA and PCL-PHEA susceptible to macrophage erosion. Note that the maximum degradation time in macrophage culture was 14 days since the viability of the cells start decreasing for longer times. Fig. 3 also shows % swelling in acetone and water of PCL-PHEA (Fig. 3A) and PLLA-PHEA (Fig. 3B). The difference between water and acetone absorption in PLLA-PHEA networks is similar to what described above for degradation in lipase solutions or in PBS.

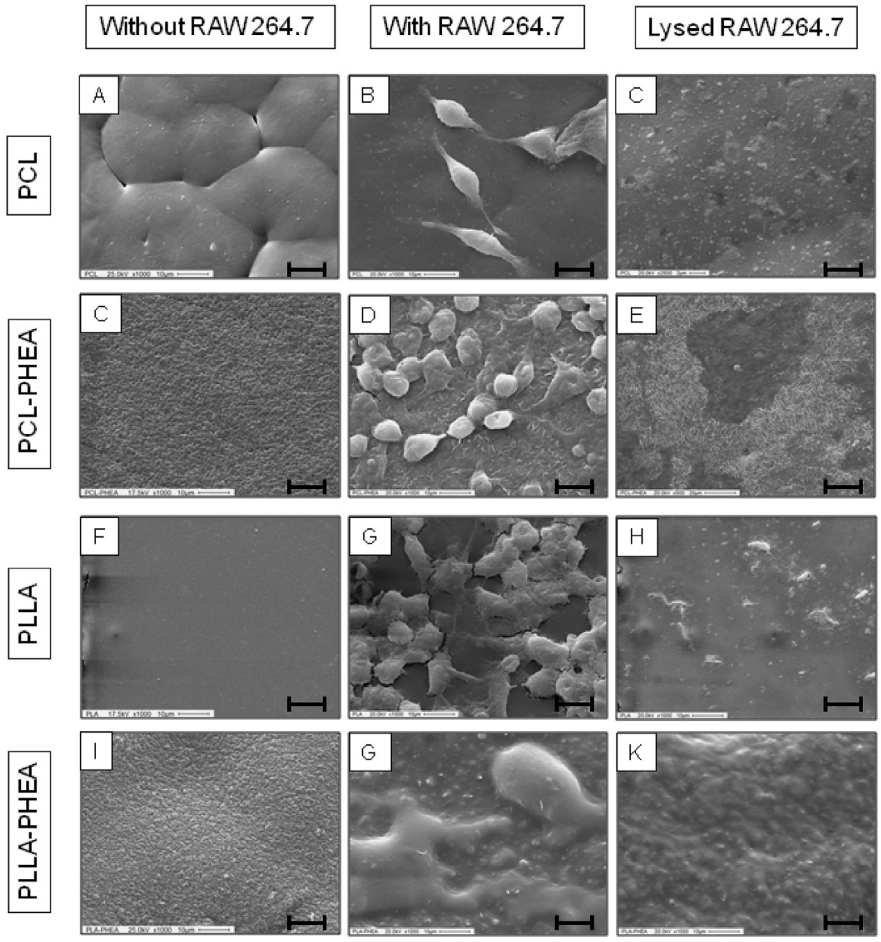


Fig. 4. SEM images of PCL, PLLA, PCL-PHEA and PLA-PHEA with RAW 264.7, without RAW 264.7 and with RAW 264.7 lysed. Scale bars correspond to 10 μm.

Absorption in acetone after 14 days degradation with RAW 264.7 was 488 ± 1% (133% absorption before degradation) while it in water was 421 ± 4%, (271% of the value before degradation). In the case of PCL-PHEA absorption in acetone after degradation with RAW 264.7 was 276 ± 2% (138% of the absorption without degradation) and in water it was only 2.64 ± 0.03%. The values found for the controls with DMEM +1% FBS were similar to those of degradation in PBS as expected.

Fig. 4 shows the SEM images of PCL-PHEA, PCL, PLLA-PHEA and PLLA, without RAW 264.7 (first column), with RAW 264.7 after 14 days of culture (second column) and 14 days of culture of RAW 264.7 and with lysed cells (third column). Macrophages adhered and growth on the surface of the hydrogels, differently, accordingly with the characteristics of the materials (Fig. 4, second column). After 14 days in culture, more macrophages were detected on the PCL-PHEA and PLLA-PHEA hydrogels, in comparison with the cells on PCL and PLLA. Even though, these effects did not directly correlate with the WCA, but it could be associated with surface topography such as the roughness and the low crystallinity.

On the other hand, the mass decrease of PCL-PHEA hydrogel after 14 days of incubation with macrophage (15.3 ± 0.1%) was about twice the degradation induced by 42 days of treatment with lipase solution (8.45 ± 1.9%). Nevertheless, the rate of PCL chain cleavage (characterized by swelling capacity) was slower than in lipase solution. This can be interpreted in the sense that macrophages mainly have an erosive action at the surface of the sample, although the effect in the core of the sample is not so important. This erosive action on the materials surface can be observed by SEM after lysing the cells with 0.1% Triton ×100 (Fig. 4, third column). In the case of PCL, the surface of the homopolymer shows clearly the structure of the spherulites. This structure is absent in the PCL-PHEA network due to the lower sample crystallinity. After macrophage culture and cell lysing, the surface of PCL-PHEA presents

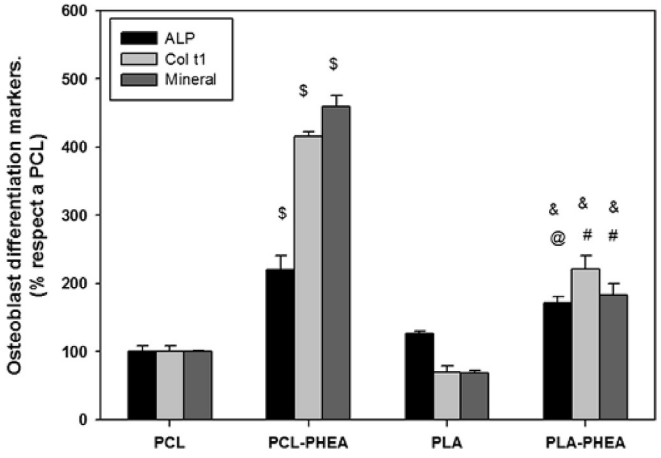


Fig. 5. Osteoblastic differentiation market of BMSCs grown over PCL and PLA films and PCL-PHEA and PLA-PHEA hydrogels. $: $p < 0.01$ respect to PCL; &: $p < 0.01$ respect to PLA; @: $p < 0.05$ respect to PCL-PHEA; #: $p < 0.01$ respect to PCL-PHEA.

rough zones that do not appear in the pure PCL samples (Fig. 4, first column). It is worth to note that these rough areas were not found in the samples degraded by hydrolysis or in lipase solution. The results in the case of PLLA are similar, although the surface erosion in the PLLA-PHEA is more uniform than in PCL-PHEA.

The fact that the erosive action of macrophages on PCL surface is very small if any was already shown by Bat et al. [7]. Notwithstanding, Mabileau et al. [33] cultivated macrophages on poly(-hydroxyethyl methacrylate) surfaces and found that the erosive effect of the cells is highly depended on the cross-linking density of the material. In this study it can be seen a significant surface effect on the PCL-PHEA block copolymer that could be due to the synergic effect of the different polymer chains, favoring macrophage growth on the material surface. On the other hand PHEA allows permeation of the polymer to water-soluble substances, without being susceptible of degradation, and cleavage of PCL chains permits further swelling of the network and mass loss.

Films made of PCL and PLLA have been proposed for the regeneration of bone [8,9,34–41]. Fig. 5 shows the osteoblastic markers of BMSCs grown in osteogenic medium over the matrixes. After 14 days ALP of the cells grown in PCL-PHEA was higher ($: $p < 0.01$) than PCL, and PLA-PHEA (&: $p < 0.01$) than PLA. Beside, ALP was higher in cells grown in PCL-PHEA (@: $p < 0.05$) than PLA-PHEA. Collagen type I production increased over hydrogels than homopolymers, also, this marker was significantly higher over PCL-PHEA than PLA-PHEA (#: $p < 0.01$). The same behavior occurs with mineral productions after 21 days in osteogenic media. The increment of the osteogenic markers could be given for the viscoelastic environment of the hydrogels after swelling. Thus, the better differentiation of BMCS was in parallel with the rugosity of the material, suggesting that the topographical characteristic could be important for driving BMCD differentiation [42].

4. Conclusions

In this study, we demonstrate that the incorporation of a small amount of hydrophilic component into the structure of hydrophobic biodegradable polyester such as PCL and PLLA makes the materials susceptible to macrophage erosion. Interestingly, in spite that pristine PLLA or PCL are not significantly eroded by macrophages cultured *in vitro*, the degradation of the networks containing PHEA is much faster in the presence of macrophages than in purely hydrolytic or enzymatic media, and much faster than in the corresponding PCL or PLLA homopolymers. On the other hand the block copolymers containing PHEA present much faster enzymatic degradation than the corresponding PCL or PLLA homopolymers although purely hydrolytic degradation rate is not so much increased. Besides this the osteogenic differentiation of bone marrow Mesenchymal Stem Cells, MSCs, cultured on these materials is studied. The Osteogenic markers was increased when MSCs was cultured on hydrogels than homopolymers.

Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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