



Osteogenic poly(ϵ -caprolactone)/poloxamine homogeneous blends prepared by supercritical foaming



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ABSTRACT

Homogeneous poly(ϵ -caprolactone) (PCL) and poloxamines (PLXs) porous blends were prepared using a supercritical carbon dioxide-assisted foaming/mixing (SFM) approach aiming to obtain cytocompatible implantable materials presenting tunable morphologies, bioerosion rates, bioactive molecules release and osteogenic features. Pure PCL, pure PLXs (T908 and T1107 varieties) and three distinct PCL:PLX 75:25, 50:50, 25:75% w/w blends, with and without the osteogenic and angiogenic bioactive molecule simvastatin were processed at constant pressure of 20 MPa and temperature of 40 °C or 43 °C, for T1107 and T908, respectively. Obtained porous blends were characterized applying a wide range of techniques and in vitro methods. Calorimetric analysis showed that hydrophilic T908 and T1107 PLXs are miscible with PCL for all tested compositions. Prepared PCL:PLX porous blends rapidly lost mass when immersed in phosphate buffer pH 7.4 due to PLXs dissolution and then went through slow and almost constant erosion rates for the subsequent weeks due to PCL slow hydrolytic degradation, which explains the rapid initial release of simvastatin and its subsequent sustained release for longer periods of time. PCL and PCL:PLX 75:25% w/w porous blends, containing or not simvastatin, showed a high cytocompatibility with SAOS-2 cells. In addition, prepared biomaterials promoted mesenchymal stem cells proliferation and their differentiation into osteoblasts. Overall, obtained results showed novel possibilities of addressing local treatment of small bone defects/fractures using highly porous PCL:PLX homogeneous blends.

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1. Introduction

The increasing prevalence of osteodegenerative diseases and of accidental bone fractures is promoting the search of affordable and improved alternatives to conventional autologous/donor grafts and bone morphogenic proteins (BMPs)-loaded scaffolds (Maia et al., 2013; Rao and Stegemann 2013; Reverchon and Cardea 2012). Research on suitable biodegradable implants that can load synthetic osteogenic pharmaceuticals and release them for a prolonged time at the injured site with minimized side-effects is attracting growing attention for the treatment of bone fractures (Han et al., 2009; Reverchon and Cardea 2012; Stakleff et al., 2013).

Simvastatin has been explored over the past years as a promoter of osteoblast differentiation and angiogenesis (Chen et al., 2010; Lo et al., 2012; Maeda et al., 2001; Mundy et al., 1999; Simões et al., 2013). This drug, which is commonly used to treat hypercholesterolemia, apparently also plays an important role in the up-regulation of BMP-2, stimulating new tissue regeneration and angiogenesis (Chen et al., 2010; Kinra and Khan, 2011; Maeda et al., 2001). However and after oral administration of safe doses, simvastatin levels at bone tissues are too low to achieve efficient therapeutic effects; therefore, the development of simvastatin-loaded implantable systems seems to be required for these therapeutic applications (Simões et al., 2013).

Recently, some poloxamine (PLX) grades, particularly BASF Tetric® 908 and 1107, showed to be the first synthetic polymers able to induce in vitro differentiation of mesenchymal stem cells to osteoblasts (Puga et al., 2012; Rey-Rico et al., 2011; Simões et al., 2013) and to promote the in vivo bone regeneration in calvarian critical defects (Rodríguez-Évora et al., 2014). PLXs are bioeliminable, amphiphilic star-shaped copolymer surfactants, that consist

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of four-arms of poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) diblocks, connected to a central ethylenediamine group. PLXs are available in a wide range of molecular weights and hydrophilic-lipophilic balances (HLB) (Alvarez-Lorenzo et al., 2007, 2010a,b) and can self-associate thus forming micelles or even gels at relatively low temperatures, including at typical biological temperatures of 33–37 °C (Alvarez-Lorenzo et al., 2010b; Chiappetta and Sosnik 2007; Cho et al., 2012; Parmar et al., 2013). Aqueous-based PLX micellar systems have been described as promising carriers of several drugs, including simvastatin (Gonzalez-Lopez et al., 2008, 2010; Simões et al., 2013). Moreover, some PLX grades showed to be miscible (in all compositions) with poly(ϵ -caprolactone) (PCL) (Puga et al., 2012), a typical biodegradable synthetic polymer having adequate features for the preparation of 3D scaffolds for hard-tissue applications (Woodruff and Huttmacher, 2010; Dash and Konkimalla, 2012).

The preparation of 3D scaffolds loaded with bioactive/signaling substances (including those which are PCL-based) is traditionally carried out by means of thermally-induced phase separation, compression and injection molding, extrusion, electro-spinning, solvent casting/particulate leaching, gas-foaming/particulate leaching, foam replication, particle sintering or rapid prototyping technologies (Liu et al., 2007; Salgado et al., 2004; Woodruff and Huttmacher, 2010). Methods based on the use of supercritical fluids (SCFs), and mainly on supercritical carbon dioxide (scCO₂), are viable and safer alternatives for the development of controlled release and tissue engineering applications, and are advantageous regarding versatility to process a large variety of polymers and labile active substances and to tune the porous structure of the scaffold (Braga et al., 2008; Davies et al., 2008; Garcia-Gonzalez and Smirnova, 2013; Kazarian, 2000; Matos et al., 2013; Natu et al., 2008; Reverchon and Cardea, 2012,b; Tai et al., 2007a,b). In general terms, the scCO₂-assisted foaming/mixing (SFM) method is based on the scCO₂ ability to drive amorphous and semi-crystalline polymers into a viscous molten state, at moderate temperatures and pressures, and which can be as well used to mix/disperse other additives (polymers, inorganics, drugs/proteins, etc.) into the polymeric materials being processed. This happens because the high pressure or supercritical phase lowers the polymer glass transition, T_g , by dissolving within polymer and by acting as a temporary plasticizer. When CO₂ is removed from the system (via pressure quenching or temperature reduction), thermodynamic instability occurs and, as a consequence, T_g increases again, re-crystallization takes place and pores are formed by nucleation and growth along with the gas removal. Morphology can be tuned by controlling different processing conditions (e.g., temperature, pressure, processing time and depressurization rate) and the processed materials will be recovered in a solvent-free state with the additives homogeneously or heterogeneously incorporated. Since low temperatures are usually employed, the method does not promote thermal degradation of labile substances (Collins et al., 2008; Fanovich and Jaeger, 2012; Jenkins et al., 2006; Kiran, 2009; Matos et al., 2013).

Following previous results (Puga et al., 2012), the main objective of this work was now to develop 3D PCL:PLX biomaterials, prepared at much lower temperatures than using the hot-melting procedure, and that may exhibit high porosity and the chemical, mechanical, morphological and biological properties suitable for hard tissue regeneration purposes. PCL and PLX (Tetronic[®] 908 and Tetronic[®] 1107) were processed by a SFM methodology, at different compositions, and with or without the simultaneous incorporation of known amounts of simvastatin. Micro- and macrostructure, erosion behavior, simvastatin release and cytocompatibility were evaluated in detail. In addition and since one additional goal of this work was to elucidate if any synergistic effect can occur on the osteogenic differentiation of

mesenchymal stem cells when combining two known osteoinductive substances (namely simvastatin and employed PLX), the in vitro promotion of alkaline phosphatase activity was also thoroughly evaluated. To the best of our knowledge, this is the first time that PCL:PLX porous blends and their mixtures with simvastatin (or any other bioactive substance) was attempted by using scCO₂, and particularly by using the SFM methodology.

2. Materials and methods

2.1. Materials

PCL (M_n 42,500 g/mol) from Sigma–Aldrich (St. Louis, MO, USA) was used in a powder form (Léonard et al., 2008). Employed poloxamines were Tetronic[®] 908 (T908, M_n 25,000 g/mol) and Tetronic[®] 1107 (T1107, M_n 15,000 g/mol) from BASF (New Milford, CT, USA). T908 was hand-grinded before use. Simvastatin (98% purity, M_w 418.57 g/mol) was obtained from AK Scientific Inc. (Union City, CA, USA). All other reagents/solvents were analytical grade.

2.2. Methods

2.2.1. PCL:PLX porous blends preparation

PCL:T908 and PCL:T1107 0:100, 25:75, 50:50, 75:25, and 100:0 w/w (total weight 1.3 g) were physically mixed in 10 mL glass vials (inner diameter 1.7 cm), with or without simvastatin (0.01% w/w = 0.1 mg/g), which were placed in a high-pressure cell, kept at a constant temperature. SFM method was carried out as previously reported (Matos et al., 2013). CO₂ was loaded into the high-pressure cell up to 20 MPa (supercritical conditions). Two temperatures were tested: 40 °C for T1107 and 43 °C for T908, according to their previously determined “melting” behavior in contact with scCO₂. The high-pressure system was kept at constant pressure/temperature conditions for 1 h with magnetic stirring (700 rpm). Then, depressurization was performed at 1 MPa/min. Obtained PCL:PLX porous blends were kept in vials and protected/kept away from light.

2.2.2. Physical and structural characterization of PCL:PLX porous blends

PCL, PLXs, and PCL:PLX porous blends (with or without simvastatin) were characterized regarding FTIR-ATR spectroscopy (Spectrum Jasco 4200 type A, Easton MD, USA; 500–4000 cm⁻¹ range, with 4 cm⁻¹ resolution); weight loss by thermogravimetric analyses (TGA; Q500, TA Instruments, New Castle DE, USA; heating at 10 °C/min from 25 °C up to 600 °C, under nitrogen flow); melting and degradation by simultaneous data thermal analysis (SDT; Q100, TA Instruments, New Castle DE, USA; heating at 10 °C/min from 25 °C up to 600 °C); T_g and crystallinity by modulated differential scanning calorimetry (MDSC; Q100, TA Instruments, New Castle DE, USA; heating at 10 °C/min from –80 °C up to 300 °C, modulated 0.32 °C every 60 s) and by dynamic mechanical thermal analysis (Tritec 2000 DMTA; Triton Technology Ltd. Nottinghamshire, UK; applying 1, 5 and 10 Hz from –150 °C up to 20 °C at 10 °C/min). Crystallinity degree was estimated as follows:

$$\text{Crystallinity}(\%) = \frac{\Delta H_m}{\alpha_{\text{PCL}} \Delta H_{m,\text{PCL}}^0 + \alpha_{\text{PLX}} \Delta H_{m,\text{PLX}}^0} \times 100 \quad (1)$$

where ΔH_m represents the sample melting enthalpy, α_{PCL} and α_{PLX} are the weight fractions of PCL and PLXs, respectively, and ΔH_m^0 is the melting enthalpy of the 100% crystalline compound, which are 142 J/g for PCL (Natu et al., 2008) and 213 J/g for PLXs (assuming that PEO blocks are the only copolymer components contributing to observed crystallinity) (Sun et al., 2004). Morphology and

porous structure analyses (optical stereo microscopy, SEM, nitrogen adsorption and helium pycnometry) were carried out as described in Appendix A.1 (Supplementary material).

2.2.3. Erosion tests and simvastatin release

Blend pieces were weighed (~50 mg) and pretreated with 5 M NaOH (5 mL), at room temperature for 72 h (Lam et al., 2009). Then, the sinking medium was replaced by phosphate buffer pH 7.4 (5 mL) and kept at 37 °C. Once a week, pieces were removed, rinsed with water, dried at 37 °C for 12 h, weighed and again immersed in the buffer. Assays were carried out in triplicate and for 4 months. For the release tests, pieces of PCL and PCL:PLX blends (45 ± 5 mg) were immersed in 2 mL of phosphate buffer pH 7.4 and kept at 37 °C under stirring (100 rpm). At pre-established time intervals, a 500 µL aliquot was withdrawn and replaced with the same volume of fresh buffer (to maintain sink conditions). Released simvastatin was quantified (as total amount and as relative content in lactone/hydroxy acid forms) using an HPLC apparatus fitted with UV–vis diode array detector (238 nm) (L-4500 Merck–Hitachi, Germany) and a LiChroCART RP-C18 (3.9 × 150 mm, 5 µM) column at 25 °C, as previously reported (Simões et al., 2013).

2.2.4. Cell studies (SAOS-2 and MSC)

Compatibility with human osteoblast-like cells (SAOS-2) was carried out as described in Appendix A.2 (Supplementary material). To test the osteoinductive properties, PCL:PLX 100:0 and 75:25 blends samples (0.9 × 0.8 × 0.1 cm, 30–50 mg), with and without simvastatin, were UV-sterilized (2 cycles, 20 min/cycle) and fixated at the bottom of 24-well plates using Silbione[®] MED ADH 4100 RTV (Bluestar Silicones, Ventura, CA USA) and a sterile cotton swap. Porous samples were allowed to react with the adhesive for 30 min and then washed three times with PBS. Mesenchymal stem cells (MSCs; StemPRO[®] human adipose derived stem cells) were maintained in MesenPRO RS[™] medium (Gibco Invitrogen, Barcelona, Spain), with 2% growth supplement, 1% glutamine and 1% penicillin/streptomycin, and then seeded (60,000 cells per well, 2 mL) over the PCL and PCL:PLX samples. Confocal microscopy images (TCSSP2, Leica Microsystems

GmbH, Mannheim, Germany) were obtained at several different times to monitor cell viability on tested samples. Calcein/propidium iodide staining (PBS:calcein:propidium iodide 98:1:1) was performed to differentiate alive and dead cells. Cell proliferation on samples surface was assessed at the same time-points, using the Quant-iT[™] Pico Green dsDNA quantification protocol (Invitrogen[™], Molecular Probes[™], Madrid, Spain). Cells on samples surface were lysed with 10 mM Tris–HCl buffer pH 7.4 containing 0.1% Triton X-100 (200 µL) and exposed to freezing (–80 °C)/thawing processes (3 cycles, 45 min/cycle). Cell lysates were then transferred to Eppendorf tubes and centrifuged (14,000 rpm, 15 min, 4 °C). Lysate samples (50 µL) were diluted with Tris–HCl (10 mM)–EDTA (1 mM) pH 7.5 buffer (100 µL) and incubated with Picogreen Reagent (50 µL) for 10 min. Dye fluorescence ($\lambda_{exc} = 480$ nm, $\lambda_{em} = 520$ nm) was measured in a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenburg, Germany). The DNA amount in each sample was calculated using a standard curve (0.0 to 1.0 µg DNA/mL). Cells seeded in osteogenic differentiation medium (10 mM β -glycerophosphate, 100 nM dexamethasone and 50 µM ascorbic acid in culture medium) were used as positive controls. Experiments were carried out in triplicate.

In parallel, MSCs were cultured in 12-well plates (10,000 cells per well, 1.5 mL) using MesenPRO RS[™] supplemented culture medium. UV-sterilized PCL:PLX 100:0 and 75:25 w/w samples (0.4 × 0.4 × 0.1; 20 ± 5 mg) were placed in the upper compartment of cell culture inserts (Millicell, Millipore Corp., Billerica MA, USA). Culture medium (0.5 mL) was added over samples until completely cover them. Cells in culture medium and cells in osteogenic differentiation medium (not in contact with samples) were used as negative and positive control, respectively. Experiments were carried out in triplicate. Cell proliferation (Quant-iT[™] Pico-Green dsDNA assay kit) and alkaline phosphatase (ALP) activity (as reported by Simões et al., 2013) were monitored at 3, 7 and 14 days.

2.2.5. Statistical analysis

The effects of PCL:PLX blend compositions on thermal properties and on MSCs proliferation and differentiation were

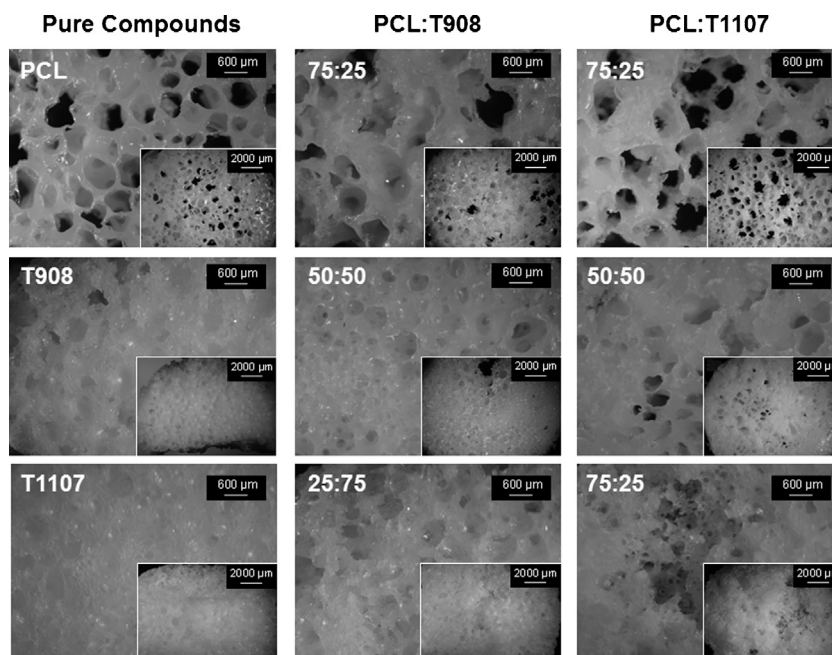


Fig. 1. Stereomicroscope images of sCO₂-processed pure PCL, T908 and T1107 samples, and of sCO₂-processed PCL:PLX blends, obtained at different magnifications (2× and 4×).

analyzed using the non-parametric Kruskal–Wallis and the Dunnett *t* post hoc tests in order to identify samples that behaved differently from the control (Statgraphics Centurion XVI software; Warrenton, VA, USA).

3. Results and discussion

3.1. PCL:PLX porous blends

Preparation of porous blends and simultaneous incorporation of additives by means of the SFM technique is a relatively economic and versatile method that avoids the use of organic solvents, harsh temperatures and additional purification steps. To the best of our knowledge, pure PLXs and PCL:PLX binary blends had not been previously processed using this methodology. Thus, several preliminary tests were performed. Although the two selected PLX grades have similar chemical characteristics, they present different molecular weights and distinct EO/PO compositional ratios; Tetronic[®] 908: M_n 25,000 g/mol, $(EO_{113}PO_{22})_2NCH_2CH_2N(PO_{22}EO_{113})_2$; and Tetronic[®] 1107: M_n 15,000 g/mol, $(EO_{60}PO_{20})_2NCH_2CH_2N(PO_{20}EO_{60})_2$. Despite the PO blocks sizes are practically the same for both PLXs, Tetronic[®] 908 has longer EO chains and therefore higher molecular weight and higher hydrophilicity than Tetronic[®] 1107. This also affects their crystallinity degree and melting/glass transition temperature and, consequently, they may behave differently upon contact and sorption with/in $scCO_2$.

Based on the visual observation through the high-pressure cell sapphire window (of the SFM apparatus), T1107 was capable to “melt” at moderated pressure and temperature conditions (~ 10 MPa and $40^\circ C$), while T908 needed a slightly higher temperature and a much higher operational pressure to achieve the molten state (~ 20 MPa and $43^\circ C$). This may be hypothesized as being the result of the relatively higher molecular weight, crystallinity and hydrophilic character of T908, which does not interact as favorably with CO_2 (a hydrophobic substance) as T1107, thus limiting CO_2 sorption, swelling and plasticization effect

(Kazarian, 2000; Kiran, 2009; Mooney et al., 1996; Sheridan et al., 2000). On the other hand, melting of PCL (M_n 42,500 g/mol) in contact with $scCO_2$ requires temperature above $35^\circ C$ and pressure above 14 MPa (Léonard et al., 2008; Matos et al., 2013,b; Salerno et al., 2011a,b; Shieh et al., 2009).

Thus and in order to easily reach PCL:PLXs blends molten states, PCL and the two PLXs were processed at a fixed pressure (20 MPa) and at two different temperatures ($40^\circ C$ for T1107, and $43^\circ C$ for T908). As observed by stereo microscopy, SFM processing of PCL:PLX blends led to highly porous materials presenting a wide range of pore sizes (Fig. 1). These visible macropores also seem to present pore sizes that are somehow dependent on the specific blend and on the relative PCL:PLX blend compositions. In addition and from a mechanical point-of-view, the greater the content in PLXs, the more brittle PCL:PLX blends were.

3.2. FTIR-ATR analysis

Due to the well-known inertness of $scCO_2$ to most chemical substances and to the specific chemical functionalities of the involved substances (Beckman, 2004), it can be reasonably hypothesized that the SFM processing of PCL:PLX blends does not chemically modify the involved polymeric materials and the incorporated osteogenic drug. As shown in Fig. 2, FTIR-ATR spectrum of pure PCL presented the typical crystalline phase peaks (carbonyl group stretching at 1727 cm^{-1} ; C–O and C–C stretching at 1293 cm^{-1}), as well as some peaks corresponding to the amorphous regions (1157 cm^{-1}) (Elzein et al., 2004; Kweon et al., 2003; Matos et al., 2013). PLX peaks are mainly due to the amorphous contribution of PPO blocks (CH_2 symmetric deformation at 1374 cm^{-1} and CH_2 wag at 1345 cm^{-1}), while the crystalline influence of PEO blocks (CH_2 wag and C–C stretch at 1350 cm^{-1}) and the combination of both PEO and PPO are located at 1280 cm^{-1} (CH_2 twist), 945 cm^{-1} (CH_2 rock and C–O–C stretch), and 1105 cm^{-1} (C–O–C stretch) (Fernandez-Tarrio et al., 2007; Su et al., 2002). Importantly, the chemical structures of PCL and PLX were not altered by $scCO_2$ during processing. Despite FTIR-ATR

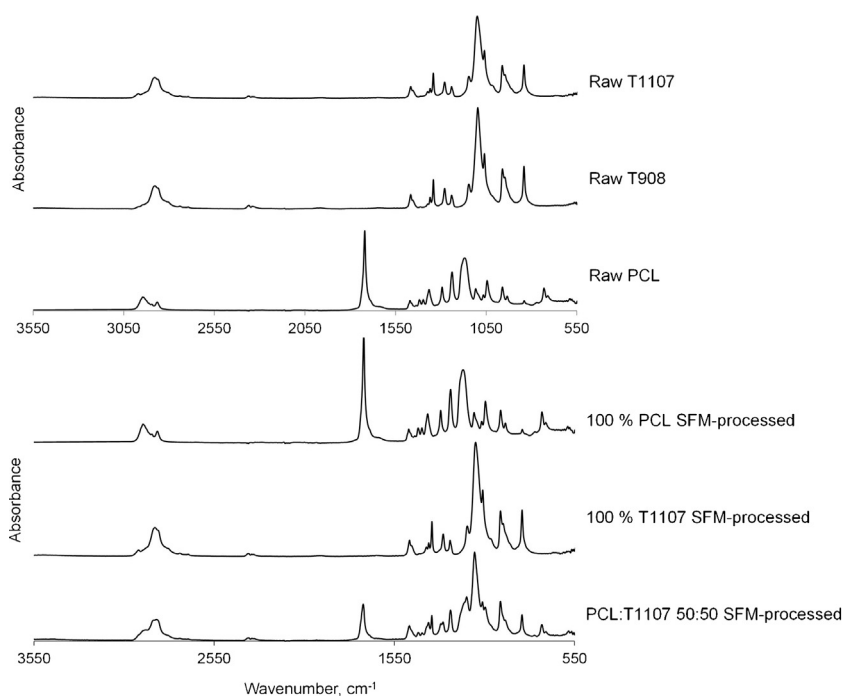


Fig. 2. FTIR-ATR spectra of non-processed (raw) polymeric materials and of SFM-processed PCL, T1107 and PCL:T1107 50:50 (processed at 20 MPa and $40^\circ C$, for 1 h, followed by 1 MPa/min depressurization). Similar results for blends were obtained when using other compositions and T908 (not presented).

Table 1

Melting temperatures and melting enthalpies (T_m and ΔH_m , respectively), crystallinity degrees (calculated applying Eq. (1)) and degradation temperatures (T_d) determined by modulated DSC and TGA, for non-processed polymeric materials (PCL, T1107 and T908). Results are presented in terms of mean values \pm standard deviations.

Non-processed materials	DSC			TGA
	T_m , °C	ΔH_m , J/g	Crystallinity, %	T_d , °C
PCL	58.5 \pm 0.3	104.2 \pm 2.6	73.4 \pm 1.8	407.6
T908	57.2 \pm 1.4	137.4 \pm 0.2	64.5 \pm 0.1	398.1
T1107	51.6 \pm 0.3	115.0 \pm 4.0	54.0 \pm 1.9	367.3

cannot be straightforwardly employed for quantitative analysis, it is interesting to observe that the ratios between the main peak areas of PCL (at 1722 cm^{-1}) and of PLXs (at 1105 cm^{-1}) (in prepared PCL:PLX blends) were found to be very similar to the weight compositions of the initial polymeric mixtures. Finally and due to the very small amounts of incorporated simvastatin, its presence could not be detected by FTIR-ATR in simvastatin-loaded PCL:PLX blends. Therefore, these spectra are not presented.

3.3. Thermal behavior

Since the SFM method always involves melting and re-crystallization processes, the thermal behavior of the starting polymeric materials and of CO_2 -processed blends was evaluated in detail by DSC, SDT, DMTA and TGA. Although the PCL, PLXs and PXL:PLXs blends behaviors at high temperatures are not relevant for this specific process and/or application, they are still presented since they may be important for other processing methods (e.g., hot-melt extrusion).

Table 1 presents melting temperatures (T_m), melting enthalpies (ΔH_m), crystallinity degrees and degradation temperatures (T_d) determined by MDSC and TGA, for non-processed PCL, T1107 and T908. Non-processed PCL and T908 presented similar T_m and T_d , while non-processed T1107 exhibited significantly lower values (Table 1). Non-processed PCL crystallinity degree (73.4%) was also higher than for non-processed samples of T908 (64.5%) and T1107 (54.0%). Crystallinity values for PLXs are in good agreement with other previous results (Puga et al., 2012) and the higher crystallinity degree observed for T908 (if compared to T1107) is probably due to its larger number of the crystalline PEO units. Similar results were obtained by SDT for non-processed PCL and PLX samples (Table 2). SFM processing increased the original (non-processed) polymeric materials melting temperatures by 2–3 °C. These results are in good agreement with what was previously reported for PCL (Kiran et al., 2008; Salerno et al., 2011a,b) and may be explained by several phase separation and thermodynamics

phenomena as well as by crystallites re-arrangement (as the result of the depressurization/desorption and/or cooling steps). Despite PLXs were never processed before using scCO_2 and SFM, similar phenomena may be involved. Moreover and since T908 has a higher crystallinity degree than T1107, these hypothetical crystallites re-arrangements should be more pronounced for T908 than for T1107. In fact, our results show that SFM processing led to an increase in melting temperature of ~ 3 °C for T908, while for T1107 this variation was of only 1 °C, which may be an additional indication that scCO_2 -induced crystallites re-arrangement are being responsible for the observed melting temperatures increase. In agreement with previous hot-melt blending results obtained by DSC (Puga et al., 2012), the SDT melting curves of SFM-processed PCL:PLX blends presented only one and well-defined peak, which is indicative of the miscibility between PCL and T908 (or T1107) for all the tested compositions. Since pure PCL presents higher T_m than T908 and T1107, the melting temperatures of the processed PCL:PLX blends were higher for samples having higher PCL proportions, and were close to that of processed PCL or in between those obtained for their components in separate. For the pure/processed polymeric materials, SDT results confirmed the already obtained modulated DSC thermal stability trends, i.e., degradation temperatures decrease in the following order: $\text{PCL} \geq \text{T908} > \text{T1107}$. As a consequence, this tendency is also reflected in blends degradation temperatures: the higher the PCL and T908 compositions, the greater the blends thermal stability and degradation temperature. DMTA results showed that the SFM processing of pure polymeric materials led to a significant decrease of T_g of PCL and T908 (from -3.1 °C and -21.7 °C down to -12.1 °C and -29.2 °C, respectively). On the contrary and considering the indicated standard deviations, T_g of T1107 did not suffer any relevant variation (it changed from -31.6 °C to -29.4 °C). This means that, after scCO_2 processing, both PLXs presented similar T_g (while their unprocessed samples presented quite different T_g values). These distinct changes in T_g values are probably the result of the different scCO_2 processing effects on T1107 and T908 (due to their distinct molecular weights, chemical structures and crystallinity degrees). Despite T908 is less hydrophobic and did not interact with scCO_2 as favorably as T1107, its higher crystallinity degree (due to its longer PEO blocks) is more affected by scCO_2 sorption, plasticization, and depressurization/desorption, which will promote a more drastic crystallites re-arrangement than in the case of T1107.

Considering the experimental standard deviations, DMTA analyses also showed that all SFM-processed PCL:T908 and PCL:T1107 blends presented T_g values between that of pure/processed PCL and those of pure/processed PLXs (Table 2). Finally and since a single and well-defined maximum of the damping parameter ($\tan \delta$) was always observed for all DMTA-tested blends (and for each

Table 2

Melting temperatures (T_m), crystallinity degrees, degradation temperatures (T_d) and glass transition temperatures (T_g) of non-processed polymeric materials (PCL, T1107 and T908), processed PCL, T908 and T1107, and processed PCL:PLX blends, determined using SDT and DMTA. Results are presented in terms of mean values \pm standard deviations.

Sample	T_m , °C	Crystallinity, %	T_d , °C	T_g , °C ^a
PCL (non-processed)	62.1 \pm 0.3	75.2 \pm 5.1	408.8 \pm 0.8	-3.1 ± 0.6
T908 (non-processed)	59.3 \pm 0.1	64.5 \pm 0.9	405.8 \pm 1.5	-21.7 ± 0.4
T1107 (non-processed)	52.6 \pm 0.1	51.6 \pm 0.5	389.0 \pm 0.4	-31.6 ± 2.8
100% PCL (processed)	64.8 \pm 0.5	64.4 \pm 1.4	408.4 \pm 0.2	-12.1 ± 1.2
100% T908 (processed)	62.5 \pm 0.3	58.8 \pm 1.3	403.4 \pm 1.7	-29.2 ± 1.1
100% T1107 (processed)	53.9 \pm 0.2	40.6 \pm 2.5	389.9 \pm 2.5	-29.4 ± 1.3
PCL:T908 25:75 (processed)	63.8 \pm 0.4	53.2 \pm 0.7	405.1 \pm 0.9	-21.0 ± 0.2
PCL:T908 50:50 (processed)	63.8 \pm 0.6	67.1 \pm 4.3	404.0 \pm 1.3	-22.6 ± 0.8
PCL:T908 75:25 (processed)	65.1 \pm 0.4	63.5 \pm 0.3	408.5 \pm 0.7	-27.1 ± 3.2
PCL:T1107 25:75 (processed)	54.8 \pm 0.4	29.0 \pm 3.2	390.6 \pm 0.5	-30.5 ± 0.3
PCL:T1107 50:50 (processed)	54.1 \pm 0.4	53.2 \pm 0.6	390.2 \pm 0.4	-27.1 ± 1.7
PCL:T1107 75:25 (processed)	63.2 \pm 0.0	58.9 \pm 0.9	407.4 \pm 0.1	-28.7 ± 2.0

^a Determined by DMTA.

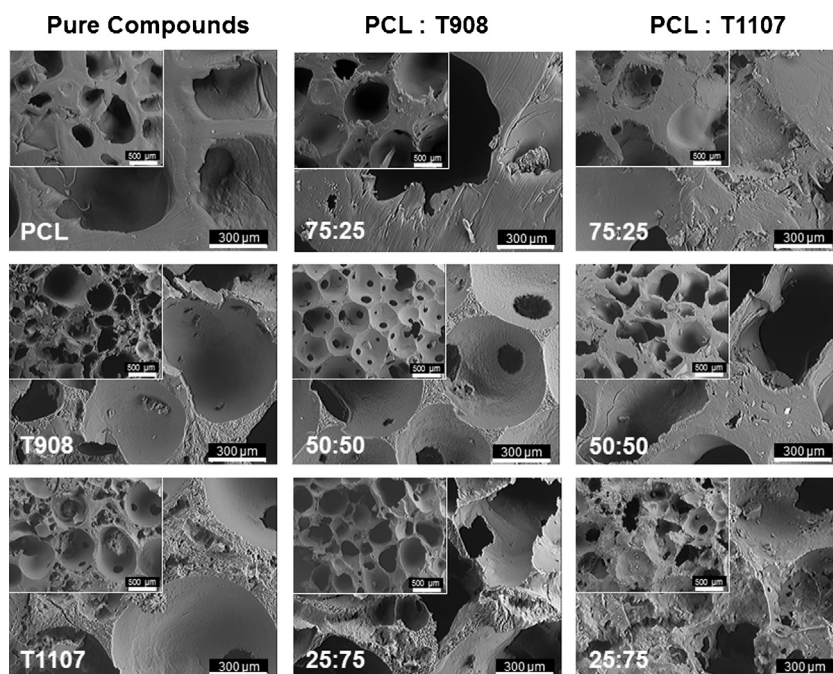


Fig. 3. SEM images of scCO_2 -processed pure PCL, T908 and T1107 samples, and of scCO_2 -processed PCL:PLX blends, obtained at different magnifications ($100\times$ and $200\times$).

employed frequency), we may conclude that processed PCL:PLX blends are miscible for all the tested compositions (Jones et al., 2012).

3.4. Macro- and micro-structural properties

PCL:PLX porous blends are characterized by pores covering a wide range of sizes, which requires complementary techniques for characterization. SEM images (Fig. 3) show that scCO_2 -processing of pure PCL led to materials having a heterogeneous pore size distribution in the 50–600 μm range (or even higher). Pore surfaces seem to be non-porous, which is probably due to “skin” formation during depressurization. Fracture cross-sections also do not show any visible porosity (at these magnifications). These observations are in good agreement with previous works (Collins et al., 2008; Fanovich et al., 2012; Jenkins et al., 2006; Matos et al., 2013b; Salerno et al., 2011a,b; Tai et al., 2007a). On the other hand, scCO_2 -processing of pure PLXs samples led not only to the same type of heterogeneous macroporosity, but also to much smaller pores (with diameters lower than 50 μm) that were visible on fracture cross-sections (higher magnifications). Again, pore surfaces seem to be non-porous (due to “skin” formation) and no relevant differences were observed between the two tested PLX grades (T908 and T1107). Overall, scCO_2 -processed pure PLXs seem to be less dense materials and to present more interconnected pore structures than scCO_2 -processed PCL.

Blending T908 and T1107 with PCL by the SFM method led to materials that present porous structures in between those of pure polymeric materials. Increasing the PLX proportion, the resulting porous morphologies become more similar to those of pure PLXs (i.e., less dense materials and more interconnected pore structures). As determined by thermal analyses, no macroscopic evidences of immiscibility were observed.

It is known that biomaterials having highly porous surfaces and interconnected pore structures are advantageous in tissue engineering and regenerative medicine applications since they enable vascularization and host/transplanted cells to interact without great restrictions, as well as they minimize barriers to the diffusion of oxygen, nutrients and metabolites between engineered and natural tissues. Conversely, closed macroporous

structures and the formation of non-porous “skins” on pore surfaces (which are formed during depressurization) are typical disadvantages of SFM processing (Mooney et al., 1996; Tai et al., 2007a). In addition to the very large and apparently interconnected macropores (between 200 μm and 2.0 mm), micropores (smaller than 2 nm), mesopores (between 2–50 nm) and small-range macropores (between 50 nm and 1 μm) are also desirable since they lead to biomaterials having high surface areas that may promote fast degradability and erosion and efficient loading, transport and release of bioactive substances (Matos et al., 2013; Salgado et al., 2004).

To gain more insight into PCL:PLX porous blends microstructure, their true densities, surface areas and pore size distributions were obtained by helium pycnometry and nitrogen adsorption experiments (Table 3). True density of scCO_2 -processed PCL was quite similar to other results found in literature (1.1–1.2 g/cm^3) (Collins et al., 2008; Fanovich and Jaeger, 2012; Matos et al., 2013). True densities of non-processed T908 and T1107 were 1.06 g/cm^3 and 1.04 g/cm^3 , respectively (as indicated by supplier); scCO_2 -processed pure PLXs showed similar values (slightly higher; probably because a different experimental setup was used). For processed PCL:PLX blends, an increase in PLX contents led to higher true densities, as expected from the higher pure true densities of processed T908 and T1107. Finally, incorporation of simvastatin did not have any effect on the true densities of the blends due to its small incorporated amounts (0.01 wt%, results not shown).

Obtained specific surface areas ranged between 0.40 and 0.65 m^2/g (Table 3). In terms of scCO_2 -processed pure samples, PLXs presented higher specific surface areas (0.527 m^2/g and 0.556 m^2/g , for T908 and T1107, respectively) than PCL (0.402 m^2/g). The surface area for processed PCL is slightly lower than values recently reported (0.52–0.91 m^2/g), which can be attributed to the use of different temperature, pressure, processing time and depressurization rates (Matos et al., 2013). As expected, blending PCL with increasing amounts of PLXs resulted in the increase of the final blend specific surface areas. No effect of simvastatin was observed.

On the other hand, accumulated pore volumes and average pore diameters results from nitrogen adsorption experiments (suitable

Table 3

True density determined using helium picnometry and surface area, pore volume and average pore diameter (<50 nm) calculated from nitrogen adsorption of the SFM-processed samples. Results are presented in terms of average value \pm standard deviation.

Sample	True density \pm SD, g/cm ³	Average pore diameter \pm SD, Å	Pore volume \pm SD, cm ³ /g	Surface area \pm SD, m ² /g
100% PCL	1.108 \pm 0.014	42.4 \pm 3.6	0.00058 \pm 0.00019	0.402 \pm 0.026
100% T908	1.134 \pm 0.005	34.4 \pm 6.5	0.00042 \pm 0.00004	0.527 \pm 0.018
100% T1107	1.134 \pm 0.004	28.6 \pm 2.8	0.00040 \pm 0.00005	0.556 \pm 0.015
PCL:T908 25:75	1.156 \pm 0.002	56.4 \pm 8.9	0.00082 \pm 0.00024	0.633 \pm 0.048
PCL:T908 50:50	1.124 \pm 0.001	35.5 \pm 2.5	0.00049 \pm 0.00001	0.575 \pm 0.021
PCL:T908 75:25	1.098 \pm 0.020	43.3 \pm 3.2	0.00049 \pm 0.00002	0.472 \pm 0.012
PCL:T1107 25:75	1.130 \pm 0.004	30.2 \pm 3.0	0.00036 \pm 0.00011	0.535 \pm 0.023
PCL:T1107 50:50	1.126 \pm 0.001	38.1 \pm 1.6	0.00054 \pm 0.00003	0.610 \pm 0.006
PCL:T1107 75:25	1.043 \pm 0.012	39.0 \pm 1.2	0.00041 \pm 0.00000	0.410 \pm 0.012

for pores in the 2–50 nm range) revealed that micropores (diameters \sim 2–3 nm) predominate in all processed samples (Table 3). Differences in obtained PCL, PLXs and PCL:PLX blends surface areas and porosities, although relatively small, may be attributed to compositional effects and to the distinct CO₂ sorption/desorption behaviors of PCL and the two different PLXs (which have different chemical structures, molecular weights and crystallinity degrees). Despite processed blends proved to be homogeneous, these systems are no longer of the binary type PCL–CO₂ or PLXs–CO₂. CO₂ interacts in a different way (and magnitude) with the polymer components of the ternary systems PCL–PLXs–CO₂. In addition, the specific interactions that may be established between PCL and each PLX (which were not present in binary systems) may also play an important role in the process. All these aspects will consequently lead to distinct CO₂ sorption, dissolution and plasticization behaviors, and to different crystallites rearrangements and required energy barrier magnitudes (upon desorption and pore formation), and consequently to distinctive final morphological properties from those of the binary systems (Kazarian, 2000; Kiran, 2009). It is also known that the greater the molecular weight and the crystallinity of the involved polymeric materials, the lower the final porosity of CO₂-processed materials will be (Sheridan et al., 2000). This also helps to explain the lower surface areas and porosities observed for PCL (which presents the higher molecular weight and crystallinity) if compared to PLXs, as well as their relative compositional effects.

3.5. PCL:PLXs dissolution and hydrolytic erosion

PCL:PLX porous blends are expected to erode and lose mass when immersed in physiological fluids, mostly due to PLXs dissolution (usually fast) and to PCL hydrolytic erosion (usually slow). Dissolution and hydrolytic degradation assays were performed by samples immersion and activation in 5 M NaOH (for 72 h at 20 °C), followed by immersion in phosphate buffer pH 7.4 at 37 °C. This procedure intends to mimic the typical in vivo erosion of PCL-based implanted biomaterials, since their biodegradation is barely affected by physiological enzymes (Lam et al., 2009). Pure PCL samples eroded very slowly and lost less than 1% of their initial mass after 4 months (Fig. 4(A) and (B)). PCL:PLX porous blends showed a rapid mass loss in the first two weeks (due to PLX dissolution), followed by a much slower mass loss due to hydrolytic degradation. No clear differences were observed between the dissolution behavior of T908 and of T1107, probably due to their similar solubilities in water. The total mass losses during the initial 1–2 weeks are almost the total observed mass loss for tested blend samples (after 4 months) and are quite similar to the total incorporated amounts of PLXs in prepared blends; for example, the final masses of PCL:T908 and PCL:T1107 75:25 blends were 83% (s.d. 7%) and 80% (s.d. 1%) of the initial sample masses (Fig. 4(A) and (B)). These remaining masses suggest some partial PLXs entrapment in less accessible regions of the non-eroded PCL

matrices (at greater depths) to the aqueous media. PCL can be considered as being hydrophobic, but their homogeneous blending with hydrophilic polymeric materials (such as PLXs) is expected to lead to a more hydrophilic system. Once PLX dissolves, the probable increase in porosity may not alter the hydrolytic degradation rate of PCL as the increased contact area with the

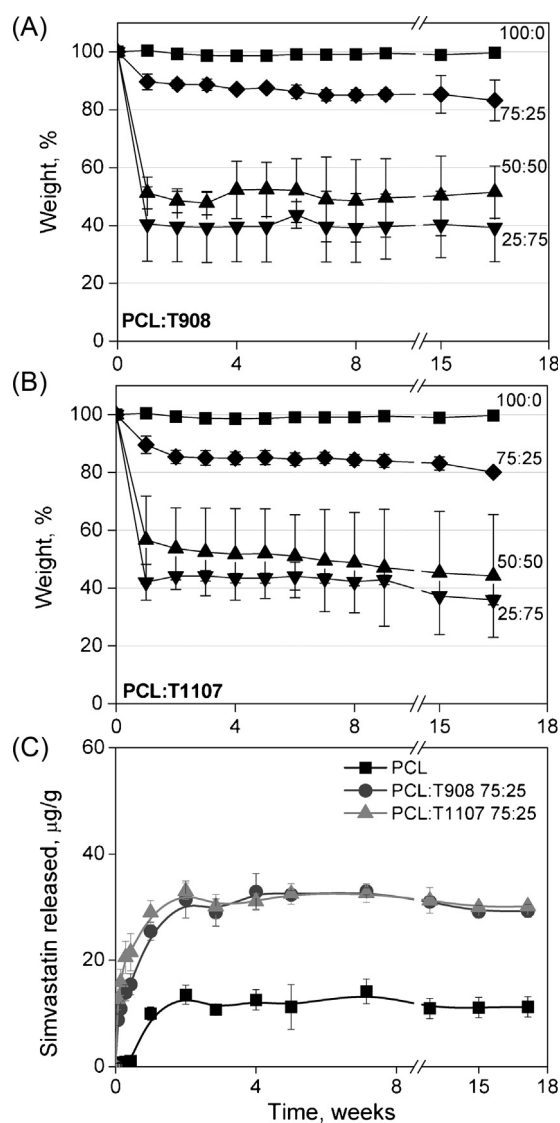


Fig. 4. Dissolution/hydrolytic behavior of sCCO₂-processed pure PCL and PCL:PLX 75:25, 50:50 and 25:75 porous blends (A for T908 blends; B for T1107). Simvastatin release into phosphate buffer pH 7.4 (at 37 °C) from sCCO₂-processed pure PCL and PCL:PLX 75:25 porous samples (C). (n = 3, error bars represent standard deviations).

aqueous medium (which may facilitate erosion) can be balanced with the facilitated removal of degradation species that may act as catalysts. Minor effects on PCL degradation rate were also previously observed for PCL:PLX blends prepared using hot-melting (Puga et al., 2012).

3.6. Simvastatin release

Simvastatin is a poorly water-soluble polycyclic drug having pH-dependent chemical structure and solubility; hydroxy acid and lactone forms have pKa 4.31 and 13.49 respectively (Pasanen et al., 2006). The anionic form of simvastatin has a much higher solubility in aqueous medium than the lactone and the free hydroxy acid forms (Alvarez-Lueje et al., 2005; Gonzalez-Lopez et al., 2010). Simvastatin also presents pH-dependent osteogenic activity since its hydroxy acid form is the only one that exhibits osteogenic activity (Simões et al., 2013). For comparison purposes, PCL:PLX 100:0 and PCL:PLX 75:25 blends were chosen for the simvastatin release studies (Fig. 4(C)).

In the absence of PLX, PCL samples exhibited a release lag period of 3–4 days. Like in erosion studies, this should happen due to the strong hydrophobic character of PCL which limits the water absorption rate/amount into its bulk. As a consequence, simvastatin release is quite slow for the initial release periods. These results also confirmed that simvastatin was homogeneously loaded into PCL and not just deposited at pore surfaces. When water finally starts to have more access into PCL deeper regions, simvastatin starts to be released, however at relatively slow rates, and reaches an almost constant release path until the end of the release period. This behavior is in agreement with the slow PCL degradation in the employed release media.

Oppositely, simvastatin release from PCL:PLX 75:25 porous blends was triggered as soon as the tested samples were put in contact with the buffer release media. This happens due to the already discussed initial faster water absorption and dissolution behaviors of the more hydrophilic PCL:PLXs blends. Simvastatin (as a hydrophobic drug) is supposed to be preferably dispersed into the hydrophobic PCL regions and the hydrophobic domains of PLXs (PPO blocks). Dissolved PLXs can form simvastatin-loaded micelles

which may help drug transport into the release media (Gonzalez-Lopez et al., 2010). After the rapid release period (first 2–3 weeks), the amounts of drug released were quite low for the rest of the release period, which is in good agreement with the slow mass loss of PCL. Despite the employed drug is different, the observed release patterns are similar to those obtained for ciprofloxacin (a hydrophilic drug) release from hot-melt PCL:PLX blends (Puga et al., 2012).

No apparent differences were observed for the simvastatin release behavior from the two tested PCL:PLX 75:25 blends. After a release period of 17 weeks, the percentages of simvastatin released were $11.28 \pm 1.92\%$, $28.65 \pm 0.61\%$ and $30.15 \pm 0.80\%$ of the initially loaded drug amounts, for PCL:PLX 100:0, PCL:T908 75:25 and PCL:T1107 75:25 blends, respectively. These results clearly show that tested PCL:PLXs blends were able to release simvastatin in a completely different way that pure PCL systems, which can be quite helpful for the proposed therapeutic application: an initially rapid drug release period in which appreciable amounts of simvastatin were released (that may stimulate new tissue regeneration and angiogenesis right after cell seeding and implantation medical procedures) followed by a much slower and sustained release over several months. Moreover, and due to the observed different dissolution/degradation behaviors, these simvastatin released amounts can be further “tuned” for the initial release period by adjusting the relative PCL:PLX compositions.

It should be referred that simvastatin concentrations (in release media) were around $0.25 \mu\text{g/mL}$ ($0.6 \mu\text{M}$) for pure PCL samples, and $0.71 \mu\text{g/mL}$ ($1.68 \mu\text{M}$) for both tested PCL:PLXs samples, which in all cases are well below the reported equilibrium (maximum) solubility in phosphate buffer pH 7.4 ($3.2 \mu\text{g/mL}$ or ca. $8 \mu\text{M}$) (Simões et al., 2013). This means that release experiments were carried out in sink conditions. Interestingly, and after 4 months of release, the simvastatin lactone form was not detected in solution, which means that the osteoinductive simvastatin hydroxy acid form was preserved in the formulations. Finally, it should also be noticed that the final simvastatin concentration in the chosen volume of release media (2 mL) for both PCL:PLXs blends (50 mg) is above the range known to be suitable to induce differentiation without causing cytotoxicity (reported to be 10 nM – $1 \mu\text{M}$)

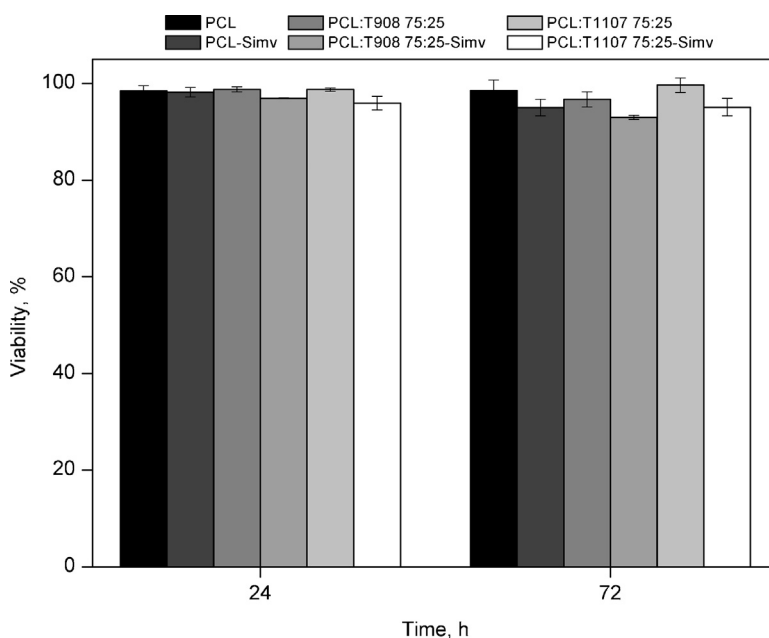


Fig. 5. Viability of SAOS-2 cells cultured in the presence of PCL and PCL:PLX 75:25 porous blends, with and without the incorporation of simvastatin ($n = 3$, error bars represent standard deviations).

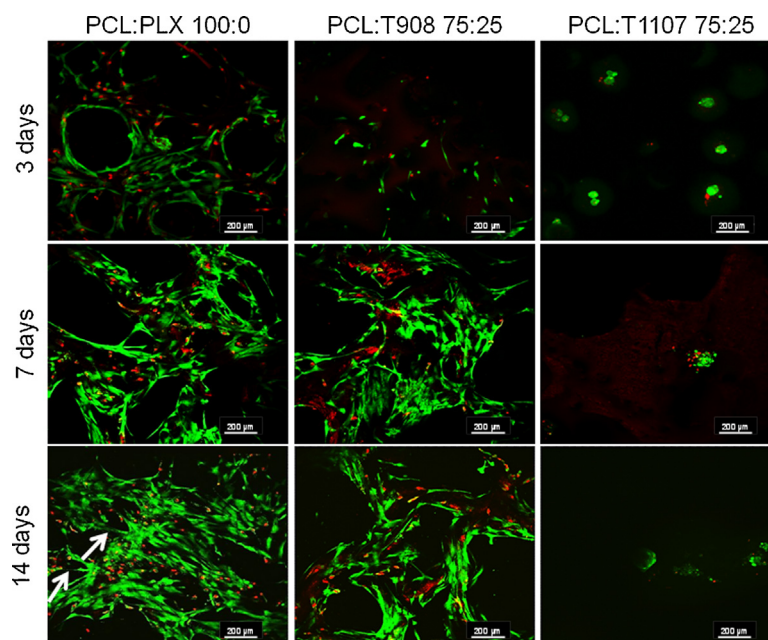


Fig. 6. Confocal microscopy images (dead/alive assay) of mesenchymal stem cells seeded on top of PCL and PCL:PLX 75:25 porous samples, without the incorporation of simvastatin. Scale bar = 200 μm .

(Jeon et al., 2007; Zhou et al., 2010; Simões et al., 2013). Release data provide useful information for the correct selection of the amount of PCL:PLX blends to be added to the cell culture medium in the subsequent experiments with mesenchymal stem cells (MSC, derived from human adipocytes).

3.7. Cells viability and osteoinductive properties

Although pure PCL, T908 and T1107 cytocompatibilities were previously assayed with several cell lines (Rey-Rico et al., 2011; Woodruff and Huttmacher, 2010), a compatibility screening with SAOS-2 cells was performed in order to elucidate if SFM processing and simvastatin release could somehow affect osteoblasts viability (Fig. 5). Only PCL:PLX 100:0 and 75:25 porous systems were tested, since an excess of PLX may prevent cell adhesion and proliferation because of the long PEO chains (Puga et al., 2012; Sosnik and Sefton, 2005). The tested systems presented high cytocompatibilities with SAOS-2 cells (at 24 h and at 72 h of contact). A slight decrease (however not very relevant) on cytocompatibility was always observed when simvastatin is incorporated (Fig. 5). This confirms that, considering the previously obtained drug release results, the concentration of simvastatin released from pure PCL and PCL:T908 75:25 and PCL:T1107 75:25 blends after 72 h (15–30 mg blend in 2 mL culture medium) of direct contact with cells was in the cytocompatibility range (Chen et al., 2010; Zhou et al., 2010), i.e. very low or undetectable simvastatin concentration for PCL and 0.23–0.46 μM and 0.32–0.66 μM for PCL:T908 75:25 and PCL:T1107 75:25 blends, respectively.

In other cell viability study, MSC were seeded on top of PCL:PLX 100:0 and 75:25 porous systems, and their viability and proliferation behavior was monitored for 3, 7 and 14 days (Figs. 6 and 7). A strong influence of the employed PLX on MSC attachment and growth was observed using confocal microscopy (Fig. 6) and dsDNA quantification (Fig. 7). Fluorescence microscopy showed that PCL and PCL:T908 75:25 samples are good substrates for the direct culture of MSC (Fig. 6), enabling good adhesion and proliferation behaviors: viable cells can be observed surrounding the pores of these two samples, and in PCL porous systems (at day 14) cells seem to be already bridging some pores (Fig. 6, signed

with arrows). This improvement in cell attachment and proliferation can be related to the formation of pores as well as the increase of surface area due to the SFM processing of the implants, compared to the ones obtained previously by hot-melting (Puga et al., 2012). On the contrary PCL:T1107 75:25 blends seem to fail to provide a good environment for cell attachment and growth, in agreement with previous reports (Puga et al., 2012; Sosnik and Sefton, 2005; Winblade et al., 2002). These results were confirmed by the evolution of dsDNA levels (Fig. 7), which indicated that pure PCL (without simvastatin) blends induced a progressive cell proliferation. For PCL:T908 and PCL:T1107 blends, the amount of dsDNA was kept constant in the first 7 days, but then decreased for PCL:T1107 blends. The incorporation of simvastatin in PCL samples led to almost constant levels of dsDNA; namely, increase in cell number is hindered by the well-known biochemical stress that the cells suffer when differentiate. The prolonged contact between MSC and simvastatin-loaded PCL:T908 and PCL:T1107 blends led to

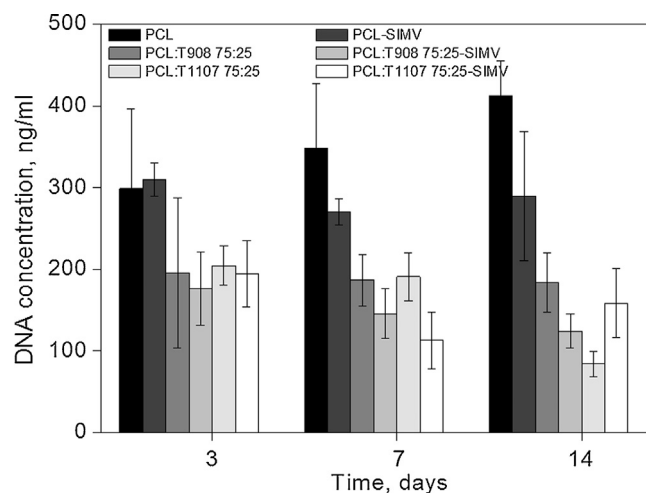


Fig. 7. Time evolution of proliferation of mesenchymal stem cells directly cultured on PCL and PCL:PLX 75:25 porous samples, with and without the incorporation of simvastatin ($n=3$, error bars represent standard deviations).

lower viabilities than in the case of PCL. These observations can be again explained by the faster release of simvastatin from PCL:PLXs samples, which affect MSC viability.

It is known that the prolonged contact (20 days) of MC3T3-E1 preosteoblastic cells with high amounts of simvastatin (1 μ M) promotes the inhibition of HMG-CoA reductase activity, and therefore of cholesterol synthesis which in turns adversely affects cell growth and activity (Jeon et al., 2007). Puga et al. (2012) observed for PCL:PLXs melt blends and ciprofloxacin that the slow drug release from PCL matrices did not affect significantly the MSCs proliferation, but for periods longer than one week the loaded and unloaded PCL:T908 50:50 matrix affects negatively the cells growth and attachment. Under in vivo conditions, the implantation site may determine the levels of simvastatin, since drug clearance from the porous system and surroundings would be mostly dictated by the physiological fluids, and thus the performance of the implant.

To gain further insight into the osteogenic and proliferation capability of the porous blends on MSCs, additional studies were carried out placing smaller pieces of PCL and PCL:PLX blends (20 \pm 5 mg) in the upper compartment of cell culture inserts, quantifying dsDNA and ALP activity after 3, 7 and 14 days of cell culture (Fig. 8). From the in vitro release experiments, simvastatin concentration in the well was estimated to be 0.3 μ M for PCL and 0.84 μ M for PCL:PLXs blends at day 14, which is below the reported toxicity limits. The dsDNA concentration (Fig. 8(A)) increased over time for all the analyzed materials, with and without simvastatin.

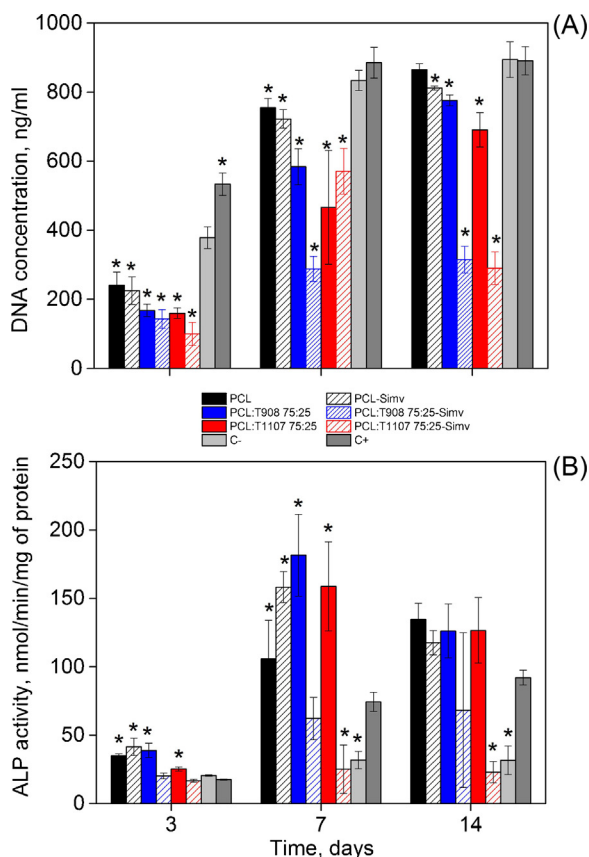


Fig. 8. Time evolution of proliferation (A) and differentiation (B) of mesenchymal stem cells cultured in the presence of PCL and PCL:PLX 75:25 porous samples (20 \pm 5 mg), with and without the incorporation of simvastatin. Results obtained in the absence of porous samples (negative control) and in osteogenic medium (positive control) are also shown. Asterisks indicate the significantly different samples ($p < 0.05$) relatively to the negative control (A) and to the positive control (B).

At days 7 and 14, cell proliferation in the presence of pure PCL blends was similar to that recorded for either negative or positive control; the incorporation of simvastatin did not modify the proliferation. However, the DNA concentration recorded for both PCL:PLXs blends was lower than that observed for pure PCL at day 7, but increased again at day 14; except for simvastatin-loaded blends for which DNA concentrations did not increase at day 14.

Regarding ALP activity (Fig. 8(B)), the opposite pattern was observed for most formulations; namely, PCL:PLX 75:25 blends without simvastatin and simvastatin-loaded PCL formulations exhibited the maximum ALP activity at day 7; which was even higher than that recorded for cells cultured in the osteogenic medium (positive control). At day 14, all the samples (except for PCL:T1107 75:25 loaded with simvastatin) led to differentiation levels higher than the positive control. This finding confirmed the osteogenic capability of simvastatin (when released at low concentration) and of PLX in separate. Simvastatin-loaded PCL:T908 75:25 blends behaved similarly to the positive control. The lowest ALP activity of simvastatin-loaded PCL:T1107 75:25 blends can be associated to the deleterious effect of T1107 on cell adhesion and of the drug on cell viability.

4. Conclusions

PCL:PLX blends with a highly porous structure can be obtained by means of $scCO_2$ assisted foaming/mixing (SFM), at relatively low temperatures ($\sim 40^\circ C$) and without using organic solvents and/or any chemical alteration/degradation in involved polymeric materials. SFM processing increased 2–3 $^\circ C$ the original melting temperatures of the components due to crystallites re-arrangement. Obtained blends showed heterogeneous pore size distribution in the 50–600 μm range and a predominance of micropores (2–3 nm) in the lower porosity range. Drug release and erosion profiles depended on the nature and proportion of PLX in the matrix. PLX promoted simvastatin release; PCL:PLX porous blends showed a faster release phase (first 2 weeks) associated to PLX dissolution and micelle formation, which in turn favored drug dissolution, followed by a sustained release of drug entrapped in the PCL network. Pure PCL and PCL:PLX blends were compatible with SAOS-2 and mesenchymal stem cells. Incorporation of simvastatin did not alter cell proliferation on pure PCL formulations, but notably enhanced mesenchymal stem cells differentiation to osteoblasts. PCL:PLX porous blends (without simvastatin) led to the highest differentiation activity, confirming the osteoinductive performance of PLX, particularly for the T908 variety. For these latter systems, incorporation of simvastatin notably decreased cell proliferation, which can be attributed to the deleterious effect that a too fast release of simvastatin may exert on cell integrity. Thus, some adjustments have to be considered in the near future regarding the possibility of decreasing either the content of PLX or of simvastatin in the blends. Overall, the results of the present work opens novel possibilities of addressing local treatment of small bone defects and fractures using easy-to-handle PCL:PLX porous blends.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijpharm.2014.12.041>.

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