Polímeros: Ciência e Tecnologia
ISSN: 0104-1428
abpol@abpol.org.br
Associação Brasileira de Polímeros
Brasil

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Polímeros: Ciência e Tecnologia, vol. 24, núm. 1, 2014, pp. 1-8
Associação Brasileira de Polímeros
São Paulo, Brasil

http://dx.doi.org/10.4322/polimeros.2014.056

In Vitro Degradation of Poly (L-co-D,L lactic acid) Containing PCL-T

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Abstract: The application of polymer-based bioresorbable temporary devices in the medical field grows continuously, and professionals from several areas act to solve problems related to body functions lost due to diseases, accidents or natural wear. Here we study the influence from poly(caprolactonetriol) (PCL-T) on the degeneration process in the copolymer poly(L-co-DL-lactic acid) (PLDLA) membrane, by producing PLDLA/PCL-T blends with 90/10, 70/30 and 50/50 relative concentrations. The data for in vitro degradation showed that PCL-T decreases the rate of PLDLA. This was obtained with the following techniques: Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Gel Permeation Chromatography (GPC) and Scanning Electron Microscopy (SEM). Therefore, it is possible to vary the membrane degradation rate by changing the blend composition, which is a tool to tailor a biomaterial.

Keywords: *Bioresorbable polymer, in vitro degradation, PLDLA/PCL-T.*

Introduction

The application of temporary bioresorbable polymer based in the medical field grows continuously, and professionals in several fields search for the solution of problems related to the restoring of body functions lost due to diseases, accidents or natural wear[1].

Among the primary bioresorbable polymers, the most important are: poly (glycolic acid) (PGA), poly (L-lactic acid) (PLLA), the copolymer poly (L-co-DL-lactic acid) (PLDLA), and poly (ε- caprolactone) (PCL)[2].

These polymers chemical properties allow hydrolytic degradation through esterefication, forming carboxy- and hydroxy-terminal groups. PLLA degradation has been found to be dependent on a range of factors, such as temperature, presence of carboxy- or hydroxy- terminal groups, molecular weight, crystallinity, purity, pH, water permeability[3]. Though biocompatible, the excessive longevity of PLLA (2 to 5 years to be reabsorbed), indicates that despite being satisfactory clinically, it is not an ideal implant material, and that improved absorbable materials need to be developed[4].

On the other hand, the use of copolymer poly (L-co-D, L lactic acid) (PLDLA) becomes attractive in several fields of application, for it does not generate highly crystalline fragments, and its reabsorption process is shorter compared to the poly (L-lactic acid) homopolymer[5]. However, in applications that require more flexible materials with a certain level of hydrophilicity, the poly (L-co-D,L lactic acid) membranes are not satisfactory for they are completely dense and rigid with high elasticity modulus, low stretching and hydrophobic character that makes the tissue/implant interaction difficult[6].

Low molecular mass molecules that act as plasticizers have been added to the polymer matrix, in an attempt to improve both the flexibility of the devices and the cellular interaction[7]. Poly(caprolactone triol) (PCL-T) has been used as a plasticizer[6,8]; it is a low molecular mass aliphatic polyester obtained from the opening of the e-caprolactone ring, presenting three OH groups in the polymer chain terminal monomer molecule[9].

This in vitro study is aimed at analysing the influence of the PCL-T concentration in the various parameters that evaluate the PLDLA membrane degradation process, especially in relation to these membranes' morphological issue, with the goal of obtaining bioresorbable devices with a greater range of possible applications in the medical field.

Experimental

Materials and methods

The polymer used in this work was poly (L-co-DL, lactic acid) (70:30) PLDLA, synthesized in the PUC/Sorocaba laboratory, using the synthesis route of polymerization by the opening of the lactic acid cyclic monomer rings with average molecular mass 265000 $g.mol^{-1}$[10] and poly (caprolactone triol) (PCL-T), chemical name 2-oxepanone, a 2-ethyl-2-(hidroxymethyl)-1,3-propanodiol polymer supplied by Solvay (CAPA 3091), with average molecular mass

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900 g.mol^{-1}. PLDLA/PCL-T samples were obtained by solvent evaporation at room temperature, in the following blends: (0/100), (90/10), (70/30), (50/50) % (m/m). PLDLA and PCL-Triol were dissolved in chloroform (Merck, Germany) and 10% (m/m) solutions were obtained, which were magnetically stirred for 12 hours, until homogenisation was complete. The solutions were poured in a glass mould (50×50×5mm) and placed in a solvent evaporation chamber for 24 hours. The films were dried in a vacuum hood for 24 hours and stored in a desiccator.

The PLDLA/PCL-T membranes in the 100/0, 90/10, 70/30 and 50/50 blends were placed in test tubes with a screw top previously sterilized with 70% alcohol, containing a phosphate buffer solution (PBS) pH 7,4 at 37 ± 1 °C, and were removed after 4, 8, 12 and 24 weeks, washed with distilled water and dried under vacuum for 48 hours. After dry, the samples were characterized using the techniques below:

Differential Scanning Calorimetry (DSC)

Samples weighing approximately 7-10 mg were heated in aluminium pans in a model MDSC291 Ta Instruments device, under N_2 purge from 25 °C to 200 °C at 10 °C/min and kept at this temperature for 5 min, being cooled at a rate of 10 °C.min^{-1} up to - 100 °C, remaining at this temperature for 5 min. The samples were then reheated to 200 °C, at 10 °C/min, and the corresponding curves were obtained.

Thermogravimetric Analysis (TGA)

Samples weighing about 20 mg were heated from 25 to 450 °C at 10 °C. min^{-1}, in a nitrogen atmosphere, using an STA 409C (NETZSCH) equipment.

Gel Permeation Chromatography (GPC)

The number average molecular weight (M_n), weight average molecular weight (M_w) and the polydispersion index (PI) were obtained in a Waters brand gel permeation chromatograph (GPC), at a temperature of 25 °C and Waters C18 column. Tetrahydrofuran (THF) was used as the mobile phase, at 20 mg.mL^{-1} concentration. The standard column used for the curve calibration was polystyrene at 10^2, 10^4 and 10^5 nm, and a Waters 410 refraction index detector.

Electron Scanning Microscopy (EMS)

The upper surface membrane samples and the fracture samples (obtained in liquid nitrogen) were metalized with gold (Sputter Coater BAL-TEC SCD 050) and analysed in an electron scanning microscope (JEOL JXA 860) operated at 10kV.

Results and Discussion

The applicability of a polymeric device is intrinsically linked both to the rate control and to the extension of its degradability, which makes the in vitro degradation study of the material a crucial ally in understanding this device's degradation process in the human body as a function of the mimicking of the physiological medium by the control of the assay conditions during the study[11].

The DSC curves related to the second heating for the PLDLA/PCL-T system in the 100/0, 90/10, 70/30 and 50/50 blends, after 0, 4, 8, 12 and 24 weeks of in vitro degradation are shown in Figure 1. The 100/0 and 90/10 blends presented similar characteristics during degradation (Figure 1a and b), where the polymer initially presented only the PLDLA Tg, an amorphous polymer characteristic, which is displaced to lower temperatures as a function of degradation time. After 24 weeks, an increase in PLDLA Tg was observed in comparison with the 12th week. In addition, the presence of crystallization and fusion peaks is observed, where the crystallization peak is narrower for the 90/10 blend. It was not possible to observe the PCL-T Tg for this compound.

The blend 70/30 (Figure 1c) presents the PCL-T and PLDLA Tg in –73°C and 38 °C respectively, and a PCL-T crystallization peak until the 8th week. PLDLA crystallization and fusion peaks are observed after 24 weeks. The PCL-T Tg is not detected from the 12th week.

For the 50/50 blend, the PCL-T Tg and crystallization temperature is observed in all degradation times; however, no PLDLA crystallization peak was observed for this blend (Figure 1d).

The analysis of the membranes in the 100/0 blend did not show Tg value variation (56 to 54 °C) until 4 weeks of in vitro degradation. However, after 8 and 12 weeks of degradation the PLDLA Tg values decreased to 42 °C and 23 °C, respectively, in comparison with the membrane that was not submitted to hydrolytic degradation.

Tg is related to the temperature at which the polymer chains acquire movement: as the material is degraded, smaller chains are formed and thus the need for a lower temperature to promote this movement. Such behaviour was observed by several authors. Baraúna[12] studied PLDLA membranes by solvent evaporation and observed a Tg decrease in relation to the in vitro degradation time, and this degradation was more evident after 18 weeks degradation. Motta and Duek (2006)[13], observed a Tg decrease in PLGA amorphous copolymer with the formation of crystals after 15 days' degradation.

An increased PLDLA Tg is observed for the 100/0 membrane in the 12 to 24 week degradation period, which ranged from 22 to 29 °C, where the material presented well-defined crystallization and fusion peaks. This increased Tg is a function of the appearance/increase in the material's crystallinity during this period, which acted to make the mobility of the polymer chains more difficult[14]. A possible explanation for the appearance of this trace PLDLA crystallinity during the degradation process was suggested in a study by Motta and Duek (2006)[13], who related the appearance of crystallinity to the decreased chain size, which contributes to a greater freedom of these segments, allowing them to crystallize under degradation conditions (37 °C, aqueous medium). The 90/10 and 70/30 blends presented a PLDLA Tg decrease similar in relation to the hydrolysis time; however, this decrease became more prominent for the 70/30 blend after 24 weeks.

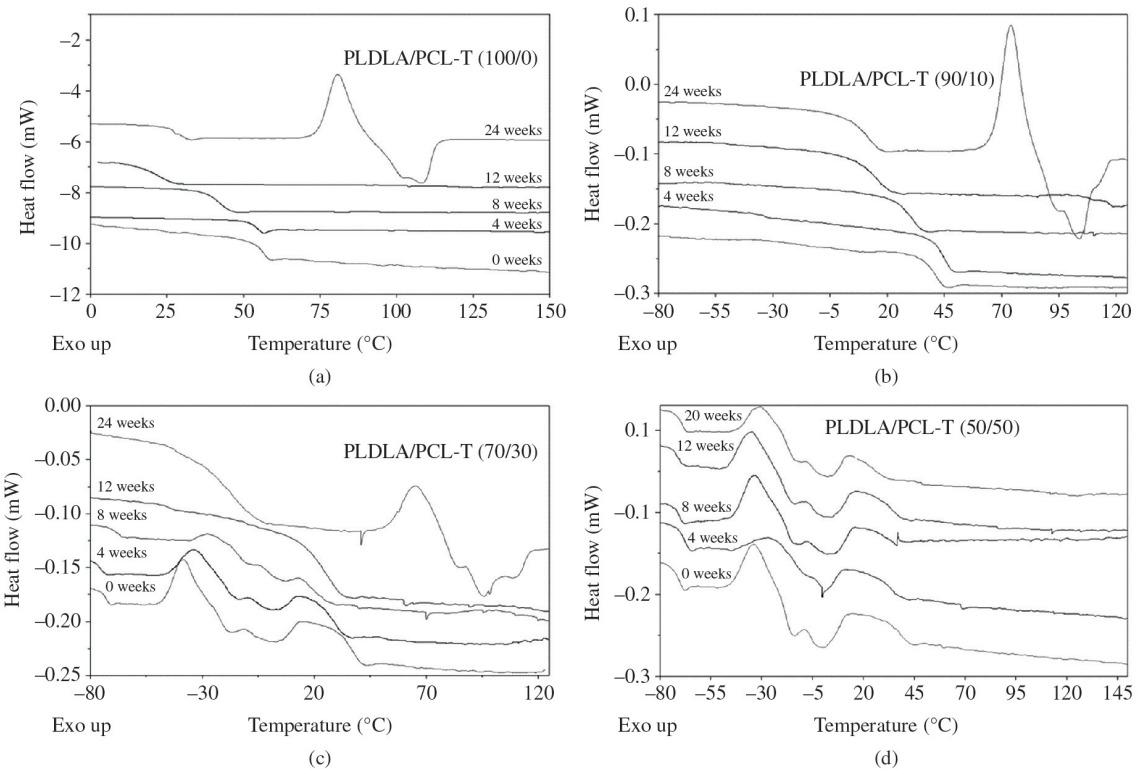


Figure 1. DSC curves (second heating) for the PLDLA/PCL-T membranes degraded in vitro in time 0, 4, 8, 12, 24 weeks. (a) 100/0, (b) 90/10, (c) 70/30 and (d) 50/50.

According to Duarte (2009)[6] the similarity in the degradation of these blends may be directly linked to the partial miscibility with PLDLA presented by both blends. The PCL-T Tg in the 70/30 blend was observed until the 8th week and was not visualized after this time (Figure 1c), which may be due to PCL-T migration or degradation.

When the PLDLA Tg values for the same blend are compared, it is observed that the membranes in the 100/0, 90/10, 70/30 and 50/50 blends present a 61, 60, 34 and 13% decrease in PLDLA Tg, respectively in up to 12 weeks, in comparison with the zero degradation time. These results indicate that the addition of the plasticizer decreases the membrane degradation velocity, particularly for the 70/30 and 50/50 blends.

This significant decrease in the 50/50 blend degradation is possibly due to the excessive amount of plasticizer, which contributes to the phase separation, increasing this materials porosity and facilitating the diffusion of degradation products by not stimulating the acid catalysis, a process that is intimately linked to the confining of these products inside the material, provoking the acceleration of the degradation process[15].

Thermogravimetric analysis (TGA) of the pure polymers (PLDLA and PCL-T) presented only one stage of mass loss, while two such stages were observed for the mixtures; the first is attributed to PLDLA and the second, to PCL-T. Table 1 shows the data obtained by TGA for all the blends as a function of degradation time.

For pure PLDLA (100/0), a decrease in the Ti and Td values was observed during the study, which confirmed the degradation process. However, it was observed that degradation for PLDLA was higher from the 4th to the 12th weeks, with 12% of degradation, as compared to that occurred between the 0th and 4th weeks, which was 4%. In the 24th week, a 2.4% decrease in the degradation percentage was observed as compared to the 12th week. This degradation behaviour was also observed for the 90/10 and 70/30 membrane blends. The lower membrane Ti from the 24th week can be due to a chain packaging that results from mobility caused by the degradation process, which allows the material's crystallization. The rate of hydrolytic degradation of PLLA decreases with increasing crystallinity in the polymer film as observed by Tsuyi and Miyauchi[16]. This increased crystallinity was confirmed by the DSC analyses.

The 50/50 blend membrane presented different behaviour in the in vitro degradation study, as shown in Table 1. PLDLA and PCL-T did not suffer significant changes in relation to their degradation. According to the literature, the degradation kinetics may be ruled by the hydrophylicity/hydrophobicity balance morphology, chemical composition and crystallinity, water diffusion, monomer solubility and diffusion, and device geometry and size[5]. Data obtained from the gel permeation chromatography (GPC) analysis as a function of hydrolysis time for the PLDLA/PCL-T membranes is shown in Table 2.

A marked decrease in the Mw and Mn values was observed for all blends after 4 weeks' degradation. From the 8th week onwards, a distinction between the blends was observed, with reduced degradation rates for higher PCL-T concentrations. The degradation process occurs through the hydrolysis of the ester bonds, giving rise to soluble oligomeric (or monomeric) products. Degradation proceeds through passive hydrolytic cleavage, reduction of weight average molecular weight (Mw) and number average molecular weight (Mn). This behaviour was marked in the PLDLA/PCL-T. membranes.

The 70/30 membrane showed differentiated behaviour in the 8th week, as shown in Table 2: the Mw values correspond to the presence of polymer,

Table 1. Data obtained by TGA for the PLDLA/PCL-T membranes as a function of in vitro degradation time. (Ti = Temperature at which the "onset" mass loss stage starts; Td = Temperature at which the curve derivative is maximum; * = difficult visualization).

PLDLA/PCL-T (100/0)	Ti PLDLA (°C)	Ti PCL-T (°C)	Td PLDLA(°C)	Td PCLT(°C)
0 weeks	340	-	354	-
4 weeks	325	-	354	-
12 weeks	285	-	342	-
24 weeks	278	-	333	-
PLDLA/PCL-T (90/10)				
0 weeks	300	-	342	344
4 weeks	318	385	342	*
12 weeks	287	373	333	381
24 weeks	275	371	308	377
PLDLA/PCL-T(70/30)				
0 weeks	292	358	317	358
4 weeks	288	356	317	358
12 weeks	245	303	274	358
24 weeks	260	376	297	391
PLDLA/PCL-T (50/50)				
0 weeks	296	375	315	370
4 weeks	276	366	315	373
12 weeks	303	383	329	400
24 weeks	280	379	313	400
Pure PCL-T	-	374	-	402

Table 2. GPC data for the PLDLA/PCL-T membranes as a function of in vitro hydrolysis time. (Mw = weight average molecular weight; Mn = number average molecular weight; IP = Polydispersity index).

Blend PLDLA/PCL-T	Mw (Dalton)	Mn (Dalton)	IP
100/0 – 0 weeks	265000	137400	1.9
100/0 – 4 weeks	47300	21700	2.2
100/0 – 8 weeks	5900	2300	2.6
100/0 – 12 weeks	3700	1400	2.6
100/0 – 24 weeks	1900	1400	1.4
90/10 – 0 weeks	285700	199200	1.4
90/10 – 4 weeks	57800	49700	1.2
90/10 – 8 weeks	5800	2600	2.2
90/10 – 12 weeks	3500	1900	1.8
90/10 – 24 weeks	1700	1400	1.2
70/30 – 0 weeks	290700	198800	1.4
70/30 – 4 weeks	67900	28700	2.4
70/30 – 8 weeks	23700	12300	1.9
70/30 – 12 weeks	7200	3700	1.9
70/30 – 24 weeks	3300	1700	1.9
50/50 – 0 weeks	231400	168800	1.4
50/50 – 4 weeks	53600	39100	1.4
50/50 – 8 weeks	49800	23700	2.1
50/50 – 12 weeks	28400	23800	1.2
50/50 – 24 weeks	27900	22100	1.3

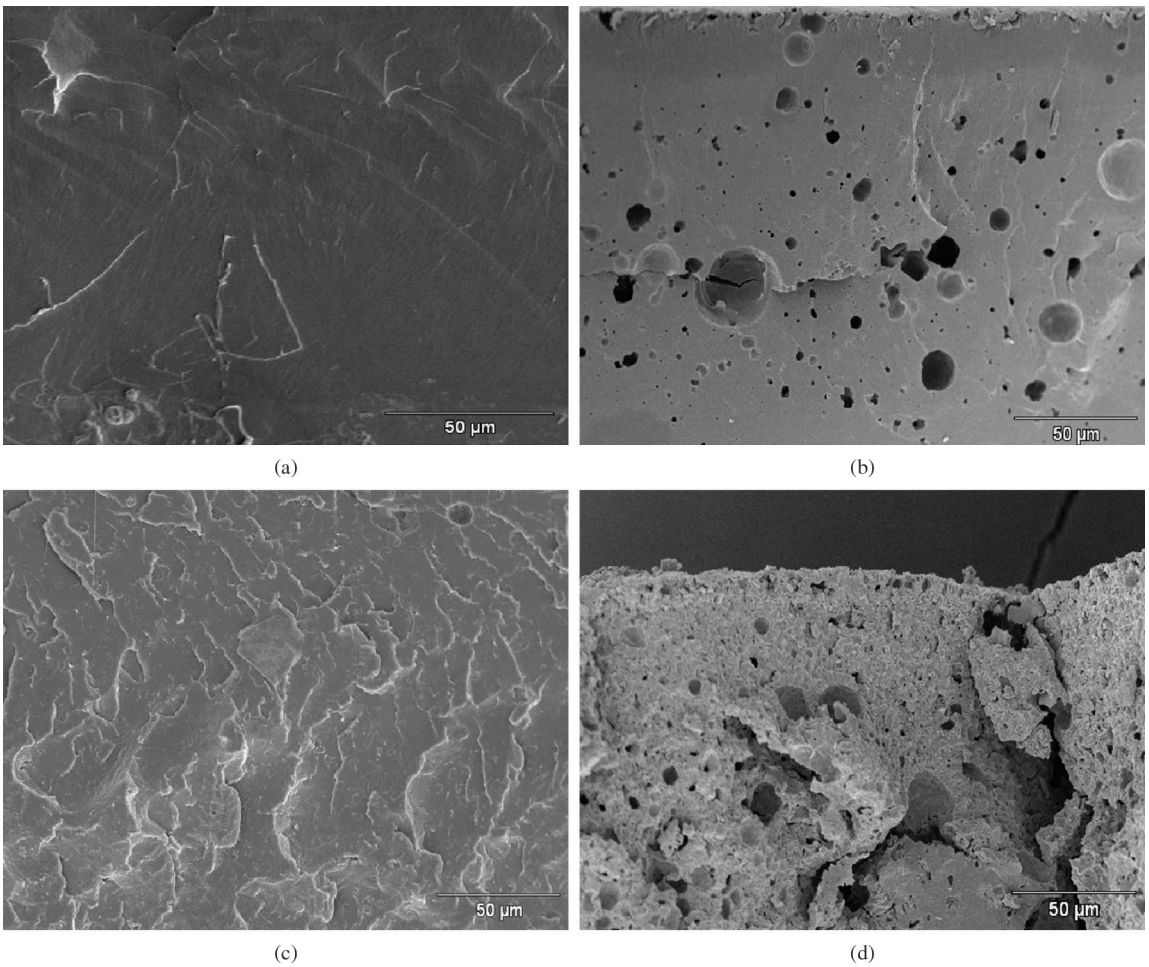
differently from the 100/0 and 90/10 blends whose values correspond only to the presence of oligomers due to high hydrolytic degradation. These results agree with the DSC and SEM analysis presented in this article. The addition of 50% PCL-T also modified membrane degradation. As discussed in the TGA analysis, the higher PCL-T concentration decreases the material's degradation velocity, as shown in Table 2.

The membrane morphologies during the degradation process were analysed using SEM. Figure 2 refers to the PLDLA/PCL-T (100/0) membrane during the study.

The membranes degraded in vitro after 4 and 12 weeks presented superficial erosions, with small pores that increased as a function of degradation time. After 24 weeks, the membranes are completely degraded, making the preparation of samples for SEM analysis difficult. The PLDLA/PCL-T (100/0) fracture degraded in vitro after four weeks' degradation (Figure 2b) was dense, with the presence of small pores when compared to the non-degraded material (Figure 2a). After 12 weeks degradation, (Figure 2c) the presence of cracks and pores located in the core of the material was observed, which proved heterogeneous degradation. After 24 weeks' degradation (Figure 2d), it was possible to observe that the material presents radially oriented degradation, which can be due to the increased PLDLA crystallinity caused by the degradation process, as observed in the DSC analyses. Pezzin et al. (2001)[17] also observed radially oriented structures, similar to spherulites, in a PLLA/PDS pin, prepared by fusion after 15 weeks degradation, where the increased crystallinity of the material can be attributed to degradation.

The in vitro degradation mechanism of the poly(α-hidroxy acids) shows that this phenomenon occurs heterogeneously, being faster in the amorphous than in the crystalline domains, and faster on the inside than on the surface, due to an acid autocatalysis.

The understanding of the preferential internal degradation is based on the in vitro study, which mimics the biological medium. The polymer is immersed in an aqueous medium where water is absorbed and the hydrolytic cleavage of the ester bonds starts, causing a molar mass decrease. In the beginning, degradation is faster on the surface than in the centre, because of the water absorption gradient. The degradation products are formed both on the surface and in the centre, but those located closer to the surface are more easily dissolved in the medium, for those located inside must diffuse



(a) (b)

(c) (d)

Figure 2. SEM micrographs of the PLDLA/PCL-T (100/0) membrane fracture obtained by solvent evaporation and in vitro degradation. a) t = 0 week, b) t = 4 weeks, c) t = 12 weeks, d) t = 24 weeks, 500x.

through the mass. Thus, the carboxylic acid grouping concentration increases more in the inside than on the surface, catalyzing the degradation[18].

The addition of 10% PCL-T totally modified the PLDLA matrix, and no changes that characterized degradation after 4 weeks' immersion in a phosphate buffer were observed. At 12 weeks, the presence of larger pores and erosions on the surface were observed; however, degradation became more marked after 24 weeks degradation, when the material was brittle, powdery and in small fragments. It was observed that after 4 weeks degradation the PLDLA/PCL-T 90/10 material had the same appearance of the non-degraded material on the fracture surface (Figure 3a), although the GPC analyses showed high molar mass loss in this period. After 12 weeks, the presence of pores and cracks was noticed, indicative of the materials degradation, which became more marked after 24 weeks (Figure 3b).

It was possible to observe that there were no signs of degradation on the upper surface of the PLDLA/PCL-T 70/30 membrane for the first four weeks immersed in a phosphate buffer solution. This behaviour was similar to that observed in the 90/10 membranes. However, the membranes degraded during 12 weeks presented cracks and pores, an evidence of the materials degradation. After 24 weeks, the material's degradation process was intensified, presenting morphology with radially oriented structures, as discussed for the 100/0 blend. On the other hand, the analysis of the fracture surface showed evidence of the degradation process only after 24 weeks, as proven by the presence of pores in the material in Figure 4d, as compared to the non-degraded material (Figure 4a).

The SEM analyses of the PLDLA/PCL-T 50/50 samples presented morphology similar to the non-degraded material for the same blend. These results indicate that the addition of 50% PCL-T slowed the degradation process. This can be seen in the micrographs of the non-degraded membrane in Figure 5a and of the membrane after 24 weeks, in Figure 5b. The presence of only a few pores in the 24-week period is the differentiation observed here.

It was observed that an increase in the PCL-T concentration decreased the PLDLA degradation, and it was possible to control the degradation rate by varying the blend, adjusting the material for a specific application. This smaller degradation velocity may be associated to the materials morphology, which is completely dense for the 100/0 blend and becomes porous after the addition of PCL-T.

Lam et al.[19], showed that porous PLLA films were more hydrolysis-resistant than dense PLLA. When the hydrolysis process of the porous and dense films were compared, it was concluded that the slowing of

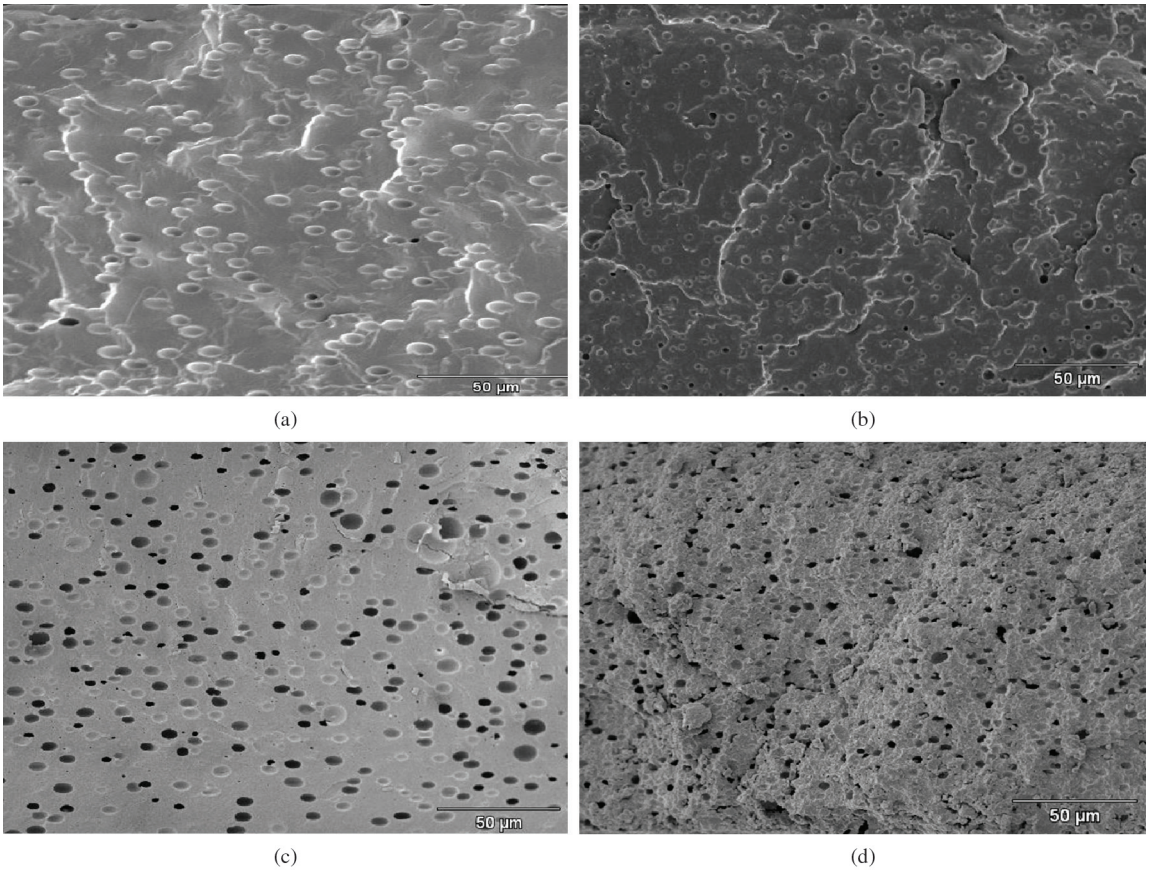
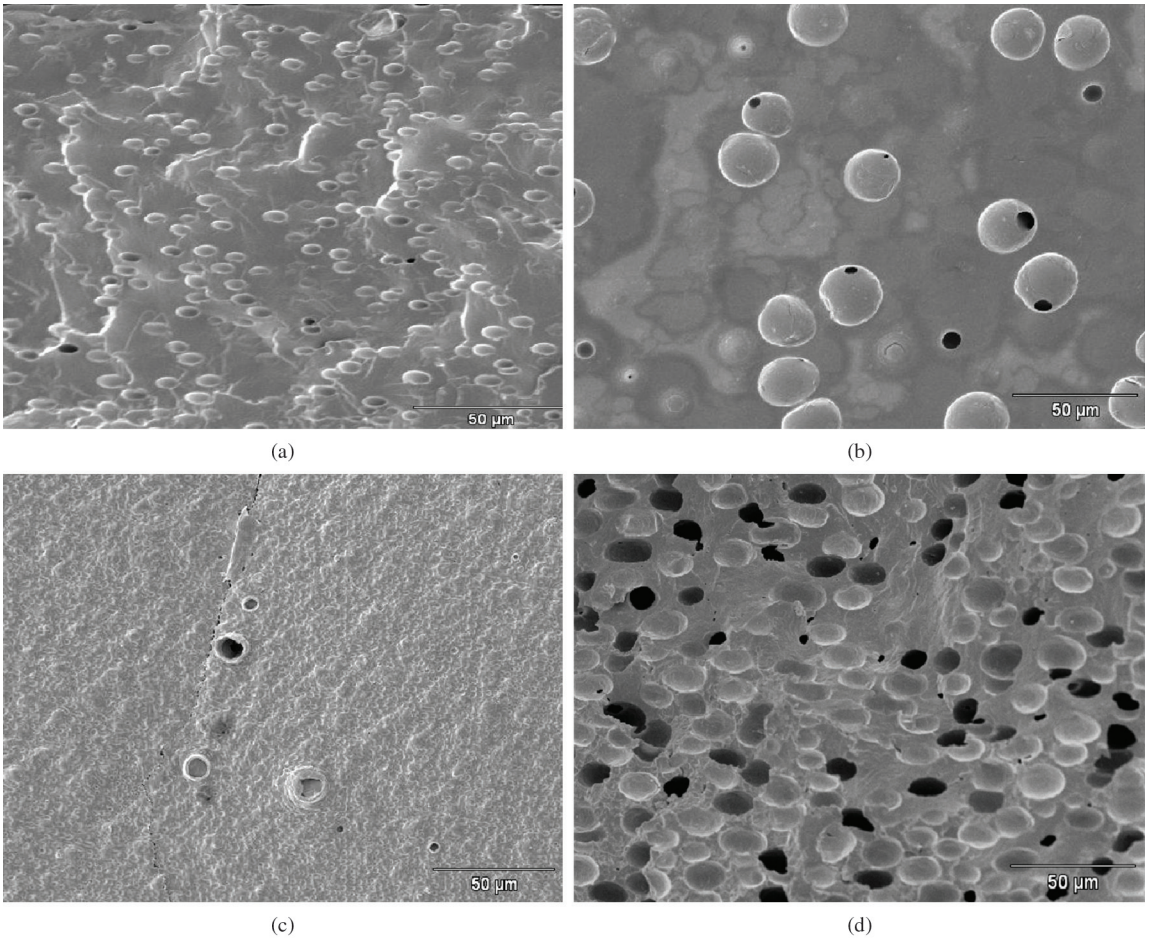


Figure 3. SEM micrographs of the PLDLA/PCL-T (90/10) membrane surface fracture obtained by solvent evaporation and degraded in vitro. a) t=0 week, b) t = 4 weeks, c) 12 weeks, and d) 24 weeks.

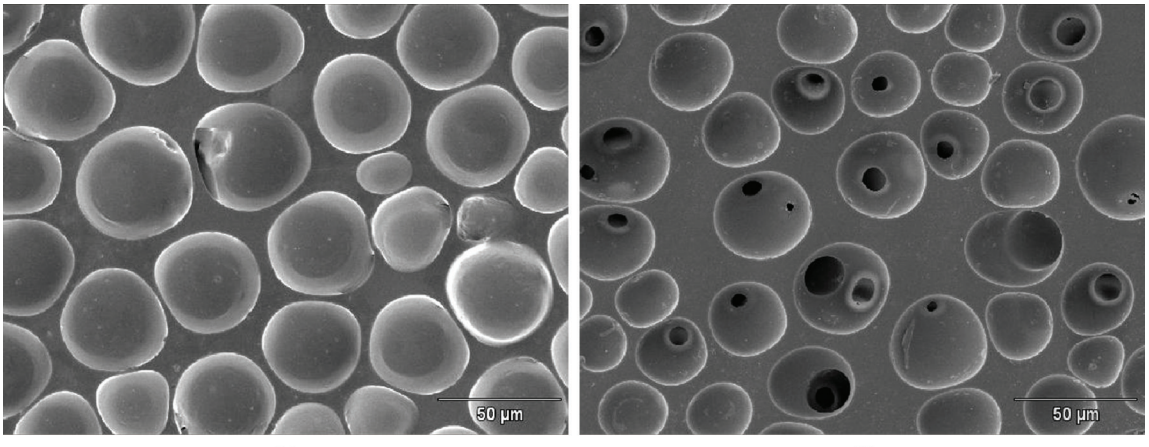
hydrolysis in porous film was due to the ease with which the degraded products were dissolved into the aqueous medium, making the accumulation of degradation products and autocatalysis difficult. The results obtained in this study were coherent with the data reported by Li[20] which reinforces the idea that the pores in the PLDLA membranes acted to decrease the degradation velocity.



(a) (b)

(c) (d)

Figure 4. SEM micrographs of the PLDLA/PCL-T (70/30) membrane obtained by solvent evaporation and degraded in vitro. a) t=-0 week, b) t = 4 weeks, c) t = 12 weeks, d) 24 weeks.



(a) (b)

Figure 5. SEM micrographs of the PLDLA/PCL-T (50/50) membrane fracture surface obtained by evaporation and degraded in vitro. a) t=0 week, b) t = 24 weeks.

Conclusions

According to the in vitro hydrolytic degradation study, it was possible to conclude that the increased PCL-T concentration decreased the PLDLA degradation velocity, which made it possible to control this copolymers degradation rate by varying its blend. Therefore, the differentiations caused by the presence of PCL-T on PLDLA could be studied and the degradation profiles could be adjusted to applications such as cell culture support, skin ulceration dressing, and guided bone regeneration.

Acknowledgments

To Unicamp and PUC/Sorocaba for the technical support and the use of their laboratories.

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Received: 06/04/13
Revised: 08/14/13
Accepted: 09/12/13