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Research Article

Investigation of PLLA/PCL Blends and Paclitaxel Release Profiles

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Abstract. Blends of poly (L-lactide) (PLLA) and poly (ε-caprolactone) (PCL) with and without paclitaxel were prepared via solution casting. DSC analysis as well as SEM analysis of the PLLA/PCL blend solution cast films showed that these blends are all phase separated.%PLLA crystallinity was found to increase with increasing PCL content (up till 15 wt.%). The PCL phase is found to homogeneously disperse in the PLLA matrix as spherical domains where the pore diameters of the PCL domains significantly increased with increasing PCL content. The degradation profiles matched with the slower degrading component PCL rather than PLLA and also increasing PCL content of the blends increased the degradation rate relatively. The increased crystallinity of the PLLA phase with increasing PCL contents confirmed that the degradation occurred through PCL phase. Cell proliferation on PLLA/PCL blends showed that all these blends were suitable for the support of cellular growth. Apoptosis assay with the paclitaxel-loaded PLLA/PCL blends showed an increase in cell death throughout 7 days of incubation where the cell death was increased with increasing PCL contents. This was attributed to the faster release of paclitaxel which was at least partially affected by the faster degradation rate at increasing PCL contents. The paclitaxel release was shown to be degradation controlled in the initial stages followed by a faster diffusion-controlled release in the later stages. These polymer blends were found to be very suitable paclitaxel release agents for which the paclitaxel release times can be altered with the composition of the blend and the film thickness.

KEY WORDS: biocompatibility; drug release; PCL; PLLA; polymer blend.

INTRODUCTION

Poly (L-lactide) (PLLA) and poly (ε-caprolactone) (PCL) are biodegradable and biocompatible polyesters that are used as implantable biomaterials such as sutures and drug delivery devices. These two polymers have been frequently studied for their use in prosthetics and in controlled drug delivery applications (1). In drug delivery application, copolymers or blends of the two polymers are studied due to their different properties which complement each other. PCL shows high permeability to small drug molecules, its degradation is slower than polylactide and does not generate an acidic environment during degradation (2,3). PLLA on the other hand shows low permeability and relatively rapid hydrolysis (1). Thus, by changing the PCL content of the blends or copolymers, the degradation rate, the permeability, and the drug release profiles of the polymers can be optimized. The degradation of both of the polymers occurs in the presence of water provoking a hydrolysis of the ester bonds. Both PLLA and PCL have a slow degradability in neutral conditions and they show a higher degradability in basic conditions than acidic ones (4). In prosthetic applications, the ductile PCL is added to the brittle PLLA and the PCL which exists as dispersed spherical domains within the PLLA matrix results in increased toughness for these phase-separated blends (5).

Although controlled delivery of certain drugs including *paclitaxel* from PLLA, PCL, or poly-L-lactide-co-caprolactone has been reported (6–8) and the PLLA/PCL blends have been analyzed in many studies for their potential use as implantable biomaterials (9–11), there has been no report on an analysis of the paclitaxel drug release from PLLA/PCL blends. Paclitaxel is a mitotic inhibitor used in certain cancer chemotherapy and in the prevention of restenosis.

In this study, films of PLLA/PCL blends (at 5, 10, 15, and 20 wt.% PCL contents) with and without paclitaxel were prepared via solution casting. The thermal transitions of these polymer blends were analyzed by differential scanning calorimetry (DSC) and the morphologies of the polymers were characterized by scanning electron microscopy (SEM). The degradation profiles of the blends at changing PLLA content have been determined and the paclitaxel release profiles obtained from the blends were analyzed in the light of these data.

In order to study cell–biomaterial interaction *in vitro*, viability of the Human Umbilical Vein Endothelial Cells

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(HUVEC) on PLLA/PCL films was determined by using CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (MTS). SEM was used to visualize cells on the surface of the films. For the evaluation of cell death, apoptosis experiment was carried out in this study.

PLLA/PCL blends were designed to be used as a controlled paclitaxel release agent which can be applied as a thin film on an implant biomaterial either as a cancer treatment agent for the targeted area or in order to prevent the tissue formation in contact with the implant.

MATERIALS AND METHODS

Materials

Paclitaxel was purchased from LC Laboratories, PCL (M_n 70,000–90,000) and PLLA (M_n ~50,400) were purchased from Sigma. HPLC-grade Dichloromethane was purchased from BDH, whereas acetonitrile and methanol were both HPLC-grade and purchased from Aldrich. PBS buffer used was purchased from PAN BIOTECH.

For the biocompatibility part of the study, gelatin and glutaraldehyde solution (25%) were obtained from Sigma-Aldrich (USA). EGM-2 Bullet kit was purchased from Lonza Clonetics (USA). DPBS with Ca and Mg 100× was the product of PAN Biotech (Germany). Trypsin EDTA 5%-10× was obtained from GIBCO (USA). MTS-CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay was purchased from Promega (USA). Cacodylic acid sodium salt trihydrate was a product of AppliChem (Germany) and Caspase-3 Apoptosis Detection Kit was purchased from Santa Cruz.

Preparation of Polymer Films

In a typical preparation, drug-containing polymer films with 0.00819 mmol (7 mg) of paclitaxel and 0.1 g of PLLA/PCL at different blend ratios (95/5, 90/10, 85/15, 80/20 *w*/*w*) were formed by casting from dichloromethane solution (10 mL), using glass molds. Dichloromethane was allowed to evaporate overnight at room temperature. Polymer films were then kept for 1 h in a vacuum oven (Memmert VO 400, Germany). Then, the films (thickness about 0.4–0.6 mm) were cut for investigation and the weights were recorded. Drug-free films were also prepared as previously described for drug-containing films.

DSC Analysis and Determination of Crystallinity of PLLA/PCL Blends

The thermal behavior of thin films of PLLA/PCL blends at different weight ratios (95/5, 90/10, 85/15, 80/20) prepared via solution casting method, was investigated by DSC (Setaram DSC 131, France). Each film sample (5–8 mg) was scanned from 25°C to 300°C at a heating rate of 5°C/min under nitrogen atmosphere. Neat PLLA and PCL film samples were also prepared via solution casting method similar to that of the preparation of the blend films and scanned under the same conditions for comparison.

Crystallinity of the PLLA phase in PLLA/PCL blends was determined from DSC analysis by measuring the integrated area under the PLLA melting peak and the PLLA crystallization peak and determining the corresponding enthalpy of melting, $dH_{m,PLLA}$, and crystallization of PLLA $dH_{c,PLLA}$. The crystallinity of PLLA for each of the PLLA/PCL blend samples were then evaluated using the $dH_{m,PLLA}$ and $dH_{c,PLLA}$ values according to the following equation:

$$x_{c,PLLA}(\%) = \frac{100 \times \left(dH_{m,PLLA} + dH_{c,PLLA}\right)}{93 \times X_{PLLA}} \quad (1)$$

where $x_{c,PLLA}$ (in percent) is the percent crystallinity of PLLA, X_{PLLA} is the weight fraction of PLLA, and 93 J/g stands for the enthalpy of fusion of PLLA having infinite crystal thickness. Each sample was analyzed twice.

SEM Analysis of PLLA/PCL Blend Films

The SEM analysis, performed using a Zeiss EVO 40 model instrument, was carried out on solution cast film surfaces to determine the porosity of the films caused by solvent evaporation process. The diameter of each pore was measured and size distribution by number intensity is presented for each sample. Films were fractured in liquid nitrogen and etched with tetrahydrofuran (THF) vapor at 50°C for 15 min and the SEM analysis was performed on both fractured and side surface of the films to determine the PCL phase structure in the blends.

Degradation of the PLLA/PCL Blend Films

Drug-free films were prepared, divided into eight pieces and the weight of each film was determined. Degradation was studied by shaking the films in Falcon tubes with 50 mL of PBS buffer (DPBS 10× PAN BIOTECH GmbH) at pH 7.4 and 37°C at a frequency of 100 strokes per minute (MaxQ™ Mini 4450, Barnstead). A fresh 50 mL of PBS buffer was changed weekly, while one of the films was removed from PBS and freeze-dried (ThermoSavant Modulyo D) overnight. Dried films were weighed and percent loss from the original piece was calculated. Each experiment was repeated three times and deviations were found to be less than 5%.

During this study, pH values of all solutions (1–8 pieces) were also recorded at predetermined time intervals and average pH values are presented. Deviations were less than 5% therefore error bars are not presented.

Biocompatibility Studies

Culture of HUVEC

Human umbilical vein endothelial cells (HUVEC) were cultured in endothelial cell growth medium (EGM-2) in T-25 flasks coated with gelatin (0.5%) and incubated in CO_2 incubator (Thermo Scientific, Hepa Class 100, USA) at 37°C, 5% CO_2.

Seeding of Cells onto Polymeric Films

The PLLA/PCL films were cut in equal pieces for *in vitro* experiments. They were sterilized in 70% ethanol for over-

night at 4°C. Then, they were washed with PBS and dried under laminar flow cabinet (Telstar, Bio-II-A, Spain).

Confluent monolayers of HUVEC were harvested by Trypsin/EDTA and the cells were concentrated by centrifugation at 1,500 rpm for 5 min and resuspended in medium for seeding. Cells were counted by Trypan blue staining. Aliquots of 20 μL of cell suspension was seeded onto the top of films placed in the 24-well plate. The matrices were left undisturbed in an incubator for 2 h to allow the cells to attach to the matrix. Then, 1 mL of medium was added into each well. Medium was changed every other day.

Proliferation of Cells on Polymeric Films by MTS

HUVEC seeded films (2.10^4 cells/mL) were incubated for 1, 7, and 14 days in the CO_2 incubator at 37°C. Cell Titer 96® non-radioactivity Cell Proliferation (MTS) assay was used to determine the cell density onto the polymer films. MTS reagent (200 μL) was added to each well of the 24-well plate and incubated for 120 min at 37°C in a CO_2 incubator. Absorbance was determined at 490 nm using an Elisa Plate Reader (Bio-Tek, EL x 800, USA). All experiments were performed three times.

SEM Analysis

Polymer-cell samples were prepared as described in the previous section. At the end of 7 and 14 days culture period, samples were fixed at 4°C in 2.5% glutaraldehyde, and 0.1 M sodium cacodylate buffer (pH 7.4) for 1 h. They were washed and refrigerated in 0.1 M of sodium cacodylate buffer (pH 7.4). Before SEM observation, samples were freeze-dried for 8 h and coated with gold. SEM was carried out in a Carl Zeiss (EVO, Germany) instrument.

Apoptosis Assay

In order to detect the cell death depending on paclitaxel eluting films, HUVECs (80,000 cells/well) were seeded into six-well plates and incubated for 2 h for cell attachment. Pre-UV sterilized paclitaxel including films were placed onto the cells. As a negative control, one group of cells were incubated only in culture medium. At the end of 3, 7, and 14 days culture period, the cells were trypsinized, washed with PBS, and re-suspended in PBS (400 μL). DEVD-AFC component of Caspase-3 Apoptosis Detection Kit (6 μL) was added to cell suspension and incubated for 15 min. After incubation, cell death was detected by flow cytometer (BD FACSCalibur, USA).

Drug Release Studies

Polymeric films were incubated in 5 mL of PBS at 37°C at a frequency of 100 strokes per minute. At predetermined time intervals, 5 mL samples were collected and each sample was replaced by an equal amount of fresh PBS. Dichloromethane (1 mL) was added to the vials to extract paclitaxel into the dicholoromethane phase. Upon phase separation, dichloromethane phase was taken and then placed in petri dishes. Dishes were left at room temperature and upon evaporation of dicholoromethane, paclitaxel was observed to recrystallize. Then, the crystallized drug was solubilized in 1 mL acetonitrile/water (1:1) solution to be analyzed by high-performance liquid chromatography (HPLC), as described below.

Analysis of Paclitaxel

The concentration of paclitaxel (λ=233 nm) was determined by an HPLC-integrated systems (Waters HPLC system). The Waters consisted of a detector (Waters 2489, UV), pump (Waters 1525), autosampler (Waters 717 plus), and degasser (Waters In-Line Degasser AF). The column was a C-18 reversed phase (X Bridge, Ireland). The two mobile phases in the gradient study consisted of 100% acetonitrile and 100% double-distilled water (0–8 min 25% acetonitrile, 8–20 min 44% acetonitrile, 20–45 min 80% acetonitrile, 45–50 min 25% acetonitrile). The flow rate was held constant at 0.5 mL/min. Each sample was injected twice and very similar results are obtained. The drug concentrations were determined by measuring peak areas, which were compared to a linear calibration curve of known standard concentrations. The correlation coefficient of the linear regression of the standard calibration curves were obtained to be greater than 0.95. Statistical calculation of quantification by the software is followed by the mean variance of least-square technique.

RESULTS AND DISCUSSION

Thermal Behavior and Crystallinity of PLLA/PCL Blends

Figure 1 shows the DSC thermograms of films of PLLA, PCL, and PLLA/PCL blends (95/5, 90/10, 85/15, 80/20 *w*/*w*) prepared via solution casting method. The DSC spectrum of neat PLLA film exhibits a glass transition temperature at around 63°C, an exothermic peak at around 91°C that stands for the crystallization of PLLA and an endothermic melting peak at 177°C. The spectra of the PLLA/PCL blends are characterized again by an exothermic crystallization peak for PLLA at around 84–87°C and an endothermic peak at 176–177°C that shows the melting of PLLA as well as an endothermic peak between 62°C and 63°C that shows the glass transition temperature of PLLA. The neat PCL film on the other hand exhibits only a melting peak at 58°C. This peak shows itself as a shoulder at around 54°C for the 85/15 and 80/20 PLLA/PCL films.

The enthalpy of melting, $dH_{m,PLLA,}$ and crystallization of PLLA, $dH_{c,PLLA}$ values with the corresponding peak temperatures and the glass transition temperature of PLLA as determined from the DSC spectra as well as the percent PLLA crystallinity, $x_{c,PLLA}(\%)$ values as determined according to Eq. 1 are listed in Table I. The melting temperature of PLLA for the PLLA/PCL blends does not exhibit a major change at different PCL contents. The PCL melting temperature, on the other hand, is not observed in the spectra of the 5 and 10 wt.% PCL films and is decreased for the 15 and 20 wt.% PCL films as compared to pure PCL film (Fig. 1). The crystallization peak of PLLA is also decreased from 91°C for pure PLLA, to 84–87°C for the PLLA/PCL blends indicating that the presence of PCL promotes PLLA crystallization (from glassy state). A decrease of the crystallization temperature of PLLA with a decrease in the enthalpy of

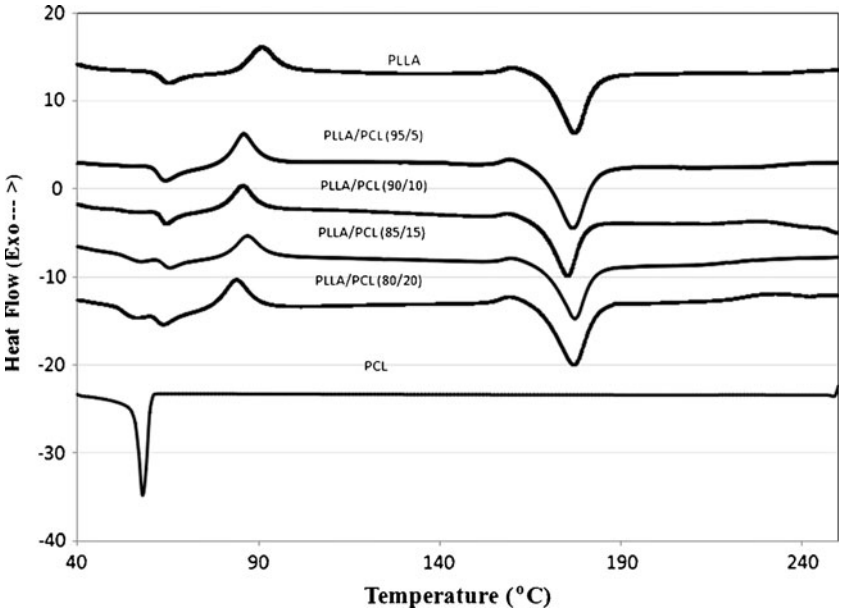

Fig. 1. DSC thermograms of films of PLLA, PCL, and PLLA/PCL blends prepared via solution casting

PLLA crystallization ΔH_{cPLLA} as compared to pure PLLA, was similarly reported for PLLA/PCL (90/10, 80/20, 70/30) melt blends prepared using a twin screw extruder (9). The melting enthalpy of PLLA ΔH_{mPLLA} as determined from the endothermic melting peak area of the PLLA matrix is decreased slightly with the introduction of PCL as compared to pure PLLA. In a similar manner, the enthalpy of PLLA crystallization ΔH_{cPLLA} decreases with increasing PCL contents. The percent PLLA crystallinity values obtained from these data on the other hand, exhibit an increase from 35% for pure PLLA to about 40% at 15 wt.% PCL content, followed by a decrease to 27% at 20 wt.% PCL content as shown in Fig. 2. The overall increase in percent PLLA crystallinity, with increasing PCL contents (until 15 wt.% PCL) indicates that the enthalpy of melting, for the blends decreases due to the decrease in the enthalpy of PLLA crystallization ΔH_{cPLLA} during the heating process. Thus, although PCL decreases the enthalpy of crystallization for PLLA during heating, the presence of PCL helps PLLA crystallization from solution. Increasing PCL content above 15 wt.% seems to disturb the formation of ordered (crystalline) structure of PLLA chains that crystallinity of PLLA starts to decrease above this content of PCL.

The presence of a well-separated melting peak for PLLA and for PCL at 15 and 20 wt.% PCL contents and the fact that PLLA melting temperature does not exhibit a major change by changing PLLA/PCL ratios show that these two polymers are phase-separated which will be later proven by the SEM analysis of the films. The decrease of the pure PCL melting temperature from 58°C to around 54°C for the PLLA/PCL 80/20 and 85/15 films and that no PCL melting peak is observed for the PLLA/PCL 95/5 and 90/10 films, however, may indicate that although the two polymers are phase-separated, there is still an interaction between them. This fact will also later be supported by the SEM analysis of the films that show the well dispersion of the PCL minor phase in the PLLA matrix. On the other hand, the percent PLLA crystallinity, values are 6.8%, 10.2%, 15.6% higher for the films with 5, 10, 15 wt.% PCL, respectively, and 23.5% lower for the 20 wt.% PCL film than that of the pure PLLA film in which PLLA molecules crystallized independently from PCL. This fact shows that PLLA and PCL are not completely phase-separated before crystallization, and that in the presence of solvent molecules, there is an interaction between them so that the phase separation occurs during the evaporation of solvent when the crystallization process takes place. If there were no interaction between the two polymers before crystallization, such a change in percent PLLA crystallinity, for the blends would not occur. Tsuji *et al.* reported a decrease in percent PLLA and PCL crystallinity and total crystallinity for PLLA/PCL solution cast films at 25%, 50%, and 75% PLLA contents and similarly attributed this decrease in crystallinity values to the interaction of the two polymers in solution before their crystallization starts (10). In the PCL content (5% to 15%) range given in this study, the interaction causes an increase in percent PLLA crystallinity. The results also indicate that although the presence of PCL enhances PLLA

Table I. DSC Results of PLLA/PCL Blends at 0–20 wt.% PCL Contents

Composition (PLLA/PCL)	$T_{m(PLLA)}$ (°C)	ΔH_m (Jg^{-1})	T_c (°C)	ΔH_c (Jg^{-1})	$T_{g(PLLA)}$ (°C)	$x_{c,PLLA}$ (in percent)
100/0	177.2±0.6	47.08±0.16	90.67±0.8	−14.77±0.53	63.1±0.6	34.74±0.73
95/5	176.7±0.6	46.80±0.3	85.92±0.6	−14.03±0.02	62.8±0.6	37.09±0.36
90/10	175.9±0.7	43.82±0.33	84.86±0.77	−11.79±0.12	63.0±0.5	38.27±0.54
85/15	176.6±0.7	43.15±0.34	87.06±0.04	−11.41±0.07	62.8±0.6	40.15±0.34
80/20	177.1±0.7	27.98±0.05	83.88±0.06	−8.21±0.27	62.0±0.8	26.58±0.42

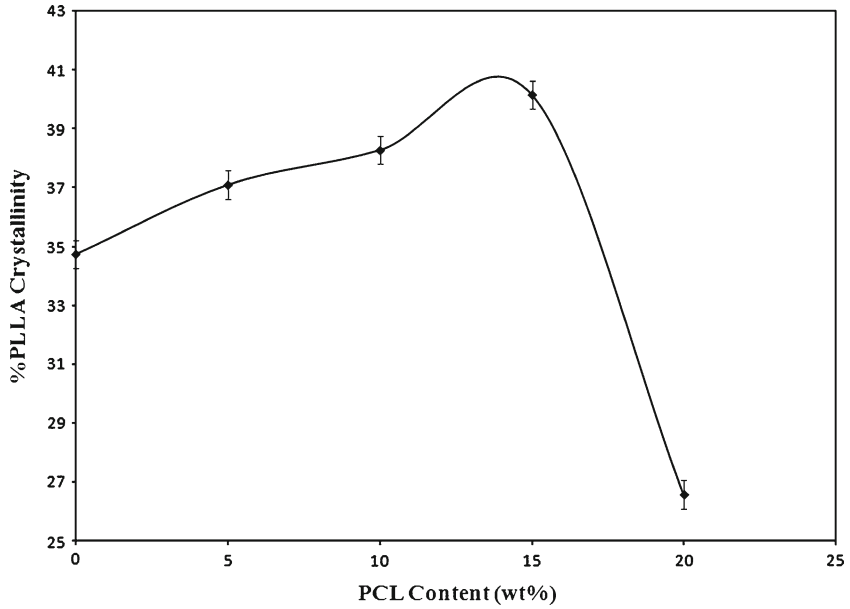

Fig. 2. Change of %PLLA crystallinity with PCL content (weight percent)

crystallization from solution, above 15 wt.% PCL content the presence of PCL domains disturbs the PLLA crystalline structure. The increase in the crystallinity of PLLA as compared to pure PLLA or the enhancement of PLLA crystallization in the presence of PCL, has been reported for the melt-blended PLLA/PCL samples in several other works (5,9,11) and is attributed to the enhancement of PLLA crystallization rate (by dynamic experiments) which likely occurs through the increase in nucleation rate (9). Although solution casting and melt-blending processes should have different effects on crystallization mechanisms of polymers, similar effects of PCL on PLLA crystallization rate may be considered for both solution cast and melt-blended PLLA/PCL blends.

SEM Analysis of PLLA/PCL and Paclitaxel-Loaded PLLA/PCL Films

Figure 3 shows the SEM images of the surface of PLLA/PCL and paclitaxel-loaded PLLA/PCL solution cast films at ×4,000 magnification. The pore size distribution of the solution cast PLLA/PCL films as determined from SEM analysis is displayed in Fig. 4. The surface SEM pictures of the PLLA/PCL solution cast films clearly show the porous morphology due to solvent evaporation process. The pore size diameters range from 1 to 5.4 μm for all PLLA/PCL blend films. SEM images of the paclitaxel-loaded films in Fig. 3b, c, and d shows that paclitaxel is well-distributed throughout the polymer films.

Figures 5 and 6 show the SEM images of the surface of the PLLA/PCL (80/20) film and the fractured surfaces of the PLLA/PCL films with changing PCL contents, each etched with THF vapor at 50°C to extract PCL. As can be seen, the PCL is dispersed homogenously in the PLLA matrix as spherical domains. Thus, as discussed in the DSC analysis of the films, these PLLA/PCL blends are phase-separated. The SEM images of the fracture surfaces of PLLA/PCL solution cast films etched with THF shown in Fig. 6 clearly indicates that the pore diameters of the PCL domains significantly decrease as the PCL content decreases from 20 to 5 wt.% PCL (from 0.5 to 2 to 0.1–0.4 μm size range). The reportedly immiscible PLLA/PCL pairs have relatively different solubility parameters thus they tend to demix from each other during solvent evaporation and coalesce in separate domains to reduce the interfacial area. The extent of coalescence in the binary PLLA/PCL blends prepared via melt blending is reported to increase with increasing concentration of the dispersed phase (9) which is in agreement with the SEM pictures of PLLA/PCL blends at various PCL contents presented in Fig. 6. Although PLLA and PCL are phase-separated, the homogenous dispersion of the minor PCL phase indicates that there is a certain extent of interaction between the two polymers. PLLA and PCL are both semi-crystalline aliphatic polyesters and the similar chemical structures of PLLA and PCL allow interpolymer polar interactions across the phase boundaries which result in the well-dispersed morphologies observed for the films as shown in Figs. 5 and 6.

The pore diameters for the etched surface of the PLLA/PCL (80/20) film (Fig. 5) are in the 0.5–1.5 μm range which are smaller than the pore diameters (1.5–5.2 μm) given for the surface of the unetched PLLA/PCL (80/20) film (Figs. 3a and 4). The same comparison is valid for the pore diameters given for the other unetched samples (Figs. 3b–d and 4) where pores are formed due to solvent evaporation, and pore diameters of the etched samples (Fig. 6b–d) where pores are formed due to extraction of PCL. This result indicates that the solvent evaporation does not necessarily occur through the PCL domains.

Degradation Studies

Among the two polymers used in this study, PLLA is predominantly amorphous, whereas PCL is a semicrystalline polymer (12). Highly crystalline polymers would be expected to degrade at a reduced rate in comparison to an amorphous or semi-crystalline polymer with a low degree of crystallinity

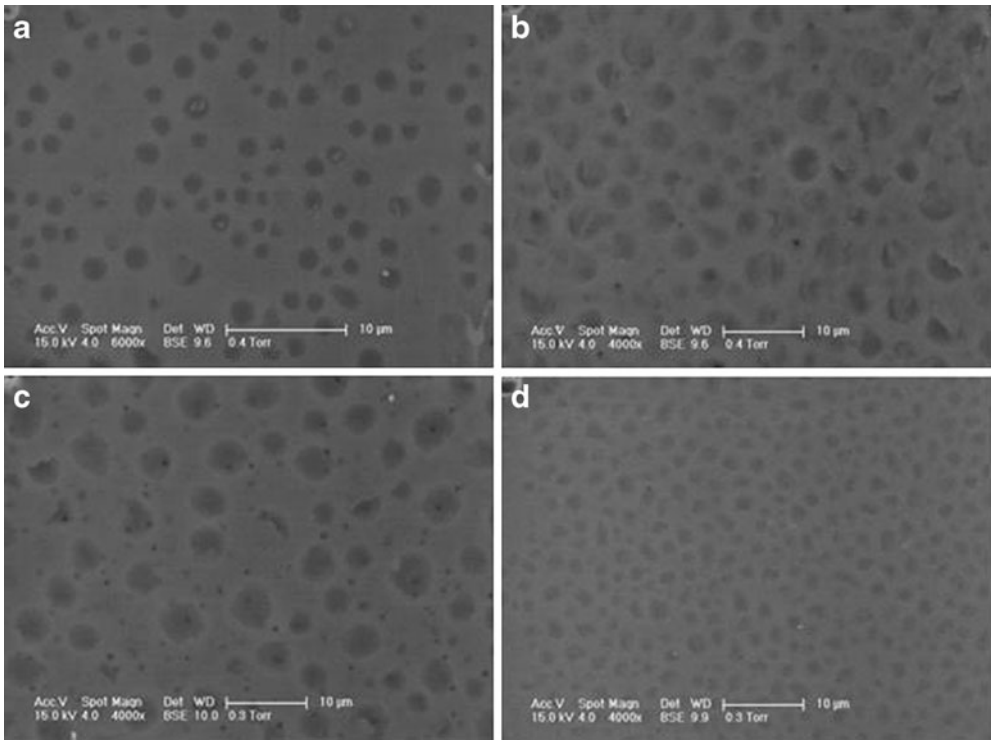

Fig. 3. SEM images of PLLA/PCL and PLLA/PCL/paclitaxel solution cast films: **a** PLLA/PCL (80/20) **b** PLLA/PCL (85/15)/paclitaxel **c** PLLA/PCL (90/10)/paclitaxel **d** PLLA/PCL (95/5)/paclitaxel at ×4,000 magnification

(12,13). This is possibly due to a preferred degradation near the chain ends, which are in most cases again situated in the amorphous part of the material (14). It can be said that the initial stage of the degradation consists of water diffusion into the amorphous regions, with random hydrolytic scission of the ester bonds. When most of the amorphous regions are degraded, hydrolytic attack progresses within the crystalline domains (15–17). As hydrolysis advances, crystalline areas are attacked and eventually degraded. Since the molecules in the amorphous region of a polymer are loosely packed, they are more susceptible to attack by reacting species or solvent than those in the crystalline region. In other words, the biodegradability of semicrystalline polymers might depend upon the amorphous structure, *i.e.*, degree of chain orientation in amorphous regions (14).

In general, PCL needs more than 110 weeks to degrade fully in a phosphate buffer (pH=7.4) (18). During the biodegradation process, the mechanical properties of PCL do not change significantly over the first 6 months, which makes selection of PCL for use as a tissue scaffold reasonable (19).

Degradation of PLLA/PCL blends are studied by direct measurement of weight loss and also by indirectly following the pH decrease due to release of acidic species which occurs due to polymer hydrolysis. The pH *versus* time and weight

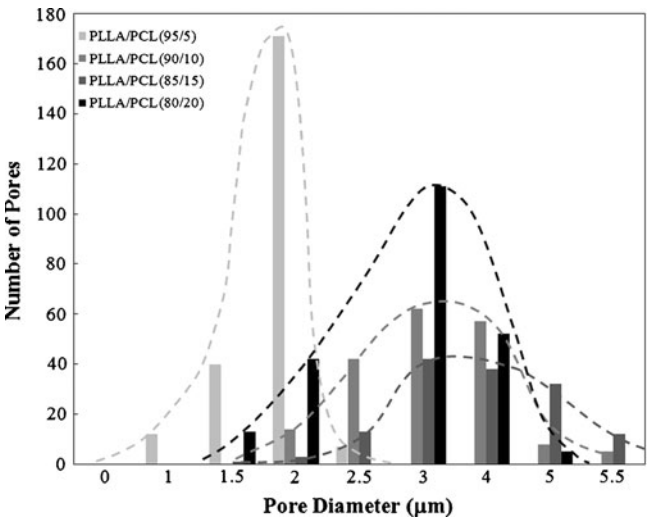

Fig. 4. Pore size distribution of solution cast PLLA/PCL films as determined from SEM analysis

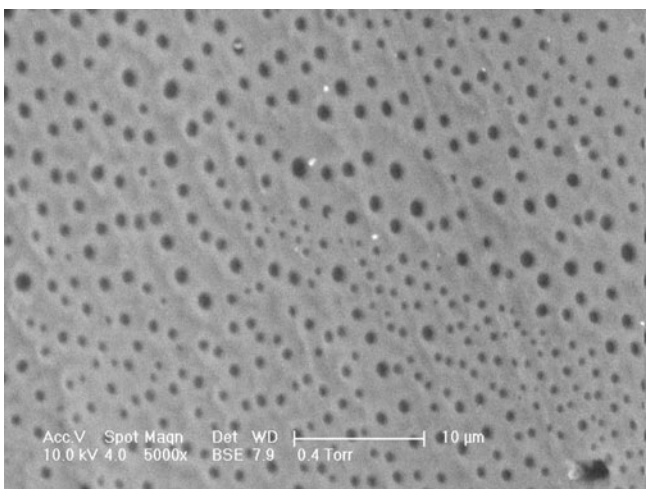

Fig. 5. SEM picture of the surface of PLLA/PCL (80/20) solution cast film extracted with THF vapor at 50°C

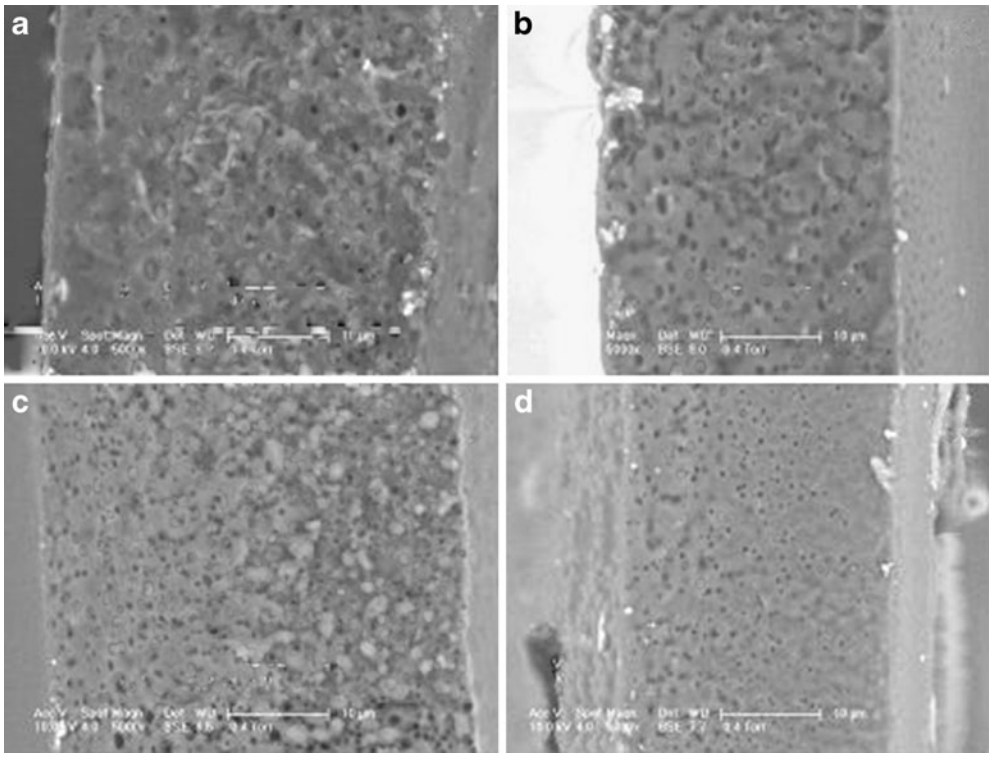

Fig. 6. SEM pictures of the fracture surface of PLLA/PCL solution cast films extracted with THF vapor at 50°C, **a** PLLA/PCL (80/20) **b** PLLA/PCL (85/15) **c** PLLA/PCL (90/10) **d** PLLA/PCL (95/5)

loss *versus* time plots showing degradation profiles of PLLA/PCL blends are shown in Fig. 7a, b, respectively. Since the degradation of PLLA and PCL release acidic species upon hydrolysis, the pH decreases from 7.4 initial value to around 6.6 for all blends. Using both methods, although similar trends were observed, weight loss studies revealed more of a distinction between different polymer blends in terms of degradation rate.

The observed degradation times are more compatible with PCL degradation possibly due to prevention of water diffusion into PLLA as a result of the presence of hydrophobic PCL (10). As can be seen in Fig. 7b, 5–25% weight loss was observed for PLLA/PCL blends from lower to higher PCL contents at the end of 55 days. Although PLLA/PCL system exhibits phase separation, the crystallization rate of PLLA could be enhanced by blending with PCL. The partial miscibility between these two polymers was suggested to cause the promotion of PLLA crystallization (20) and this is supported by DSC results in this study.

If degradation were to take place through PLLA, as crystallinity increases with PCL content, degradation rate would be expected to decrease with increasing PCL content. Our data, on the other hand, exhibits a clear increase in degradation rate as PCL content increases which suggest that the degradation of the blend is predominantly taking place though PCL domains. It should also be noted that the overall degradation time is more comparable with PCL degradation. When PLLA is alone, PLLA is said to form sperulites (10) and that PCL disturbs the morphology of these sperulites when blended with PLLA. In all the blends used in this study, PLLA is the matrix polymer. When the concentration of PCL is low, the more hydrophobic PCL will initially have a tendency to preferentially expose itself to air or PLLA surface to minimize its contact with the more hydrophilic PLLA. This hydrophobic effect eventually results in PCL domain formation, leading to phase separation of the two polymers. Thus, PCL acting like a surface-active agent, residing itself on the interfaces, is likely to retard the water penetration into the amorphous regions of PLLA, leading to slow degradation times. As the concentration of PCL increases in the blend, PCL domains get larger and due to surface/volume ratio, contact surface between PCL/PLLA gets smaller, water access to PLLA may be facilitated resulting in enhancement of degradation in the PLLA phase in addition to PCL degradation which increases the overall degradation rate.

Biocompatibility Studies

The biocompatibility of the polymer is the most significant criteria and concerning biocompatibility, the most desirable biodegradable polymer is PLLA which has been used in numerous studies of tissue engineering and biomedical applications (21,22). Cell proliferation tests of PLLA have proven that the polymer does not cause cytotoxicity and it is highly biocompatible. However, these properties are directly affected with the molecular weight and crystallinity of the polymer. PCL is another biodegradable polymer that is used for many applications in the biomaterials field and a number of drug delivery devices (23). It is suitable for controlled drug delivery due to a high permeability to many

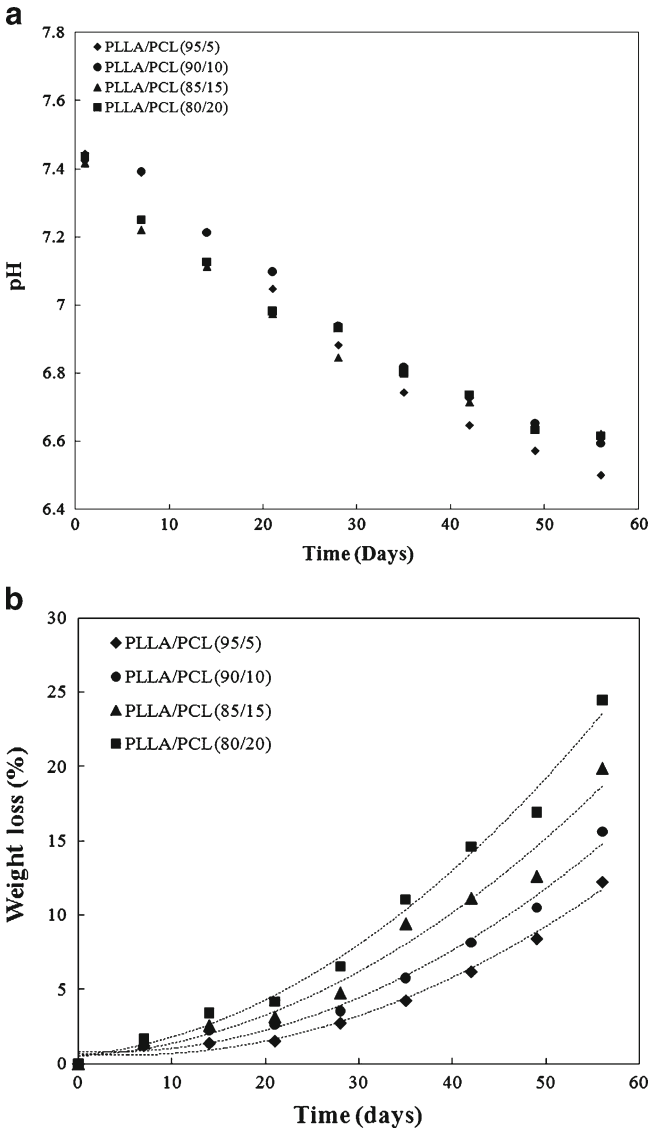

Fig. 7. Degradation of PLLA/PCL polymer blends following **a** pH decrease **b** weight loss with time

drugs, excellent biocompatibility, and its ability to be fully excreted from the body once bioresorbed.

Proliferation of Cells on Polymeric Films by MTS Assay

Different polymeric blends of PLLA/PCL (95/5, 90/10, 85/15, 80/20 *w*/*w*) prepared via solution casting were seeded with HUVECs and tested by MTS assay in order to determine the biocompatibility of the films.

Figure 8 represents cell proliferation on PLLA/PCL blends. The results showed that all of the polymeric films almost have the same cell number at the defined time intervals. Cell growth rates of the PLLA/PCL blends were almost the same with the control (OC). Cell number in all blends and control samples increased from 20,000 to 150,000–200,000 cells/mL at the end of 14 days of incubation.

Ajami-Henriquez *et al.* (24) reported similar results for *in vitro* cellular response to the different polymeric films including PLLA/PCL blends in terms of initial adhesion,

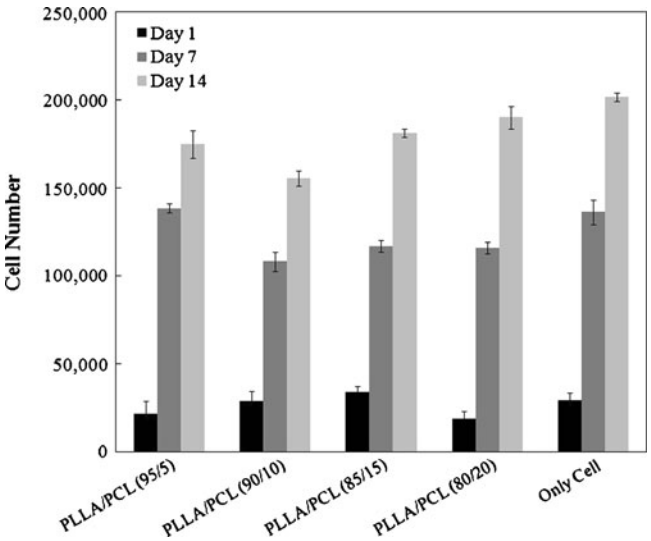

Fig. 8. HUVEC cell growth determination on PLLA/PCL films with different compositions at the end of 1, 7 and 14 days of incubation by MTS assay. OC samples do not contain PLLA/PCL films. Initial cell number was 20,000 cells per well

proliferation, morphology, and cell migration by using monkey (VERO) immortalized cell line and primary rat calvaria osteoblasts. The results indicated that all materials tested were suitable for the support of cellular growth.

SEM Analysis

HUVECs were seeded on to polymeric films and the morphologies of cells, polymers, and cell–polymer interactions were analyzed by SEM in order to observe cell presence and cell alignment on films.

Figure 9 represents cell–polymer relationship of cell-seeded PLLA/PCL films with different compositions throughout 14 days of incubation. In all blends, cell morphologies became more aligned and flattened on the films as time passes.

It was also reported in the literature (24) that different molecular weights and structures of PLLA or PCL did not affect the attachment behavior of rat calvaria osteoblasts in blends or copolymers significantly. These data also support the SEM results in the present study.

Apoptosis Assay

In order to determine the apoptotic effect of paclitaxel release from different compositions of PLLA/PCL films, apoptosis assay was performed. The cells were incubated with drug-loaded PLLA/PCL films for 1, 4, and 7 days. Flow cytometry histograms, apoptosis ratios of HUVEC due to paclitaxel elution from all PLLA/PCL blends at days 1, 4, and 7 are given in the Electronic Supplementary Material.

Figure 10 summarizes all apoptosis data. It was observed that there was an increase in cell death in all blends of PLLA/PCL films throughout 7 days of incubation, the paclitaxel-loaded films exhibiting a higher cell death than unloaded polymer films as expected.

According to the results, cell death was increased as PCL ratio increases in paclitaxel-loaded blends. In the paclitaxel-loaded 80/20 PLLA/PCL blend, nearly 53% decrease in cell

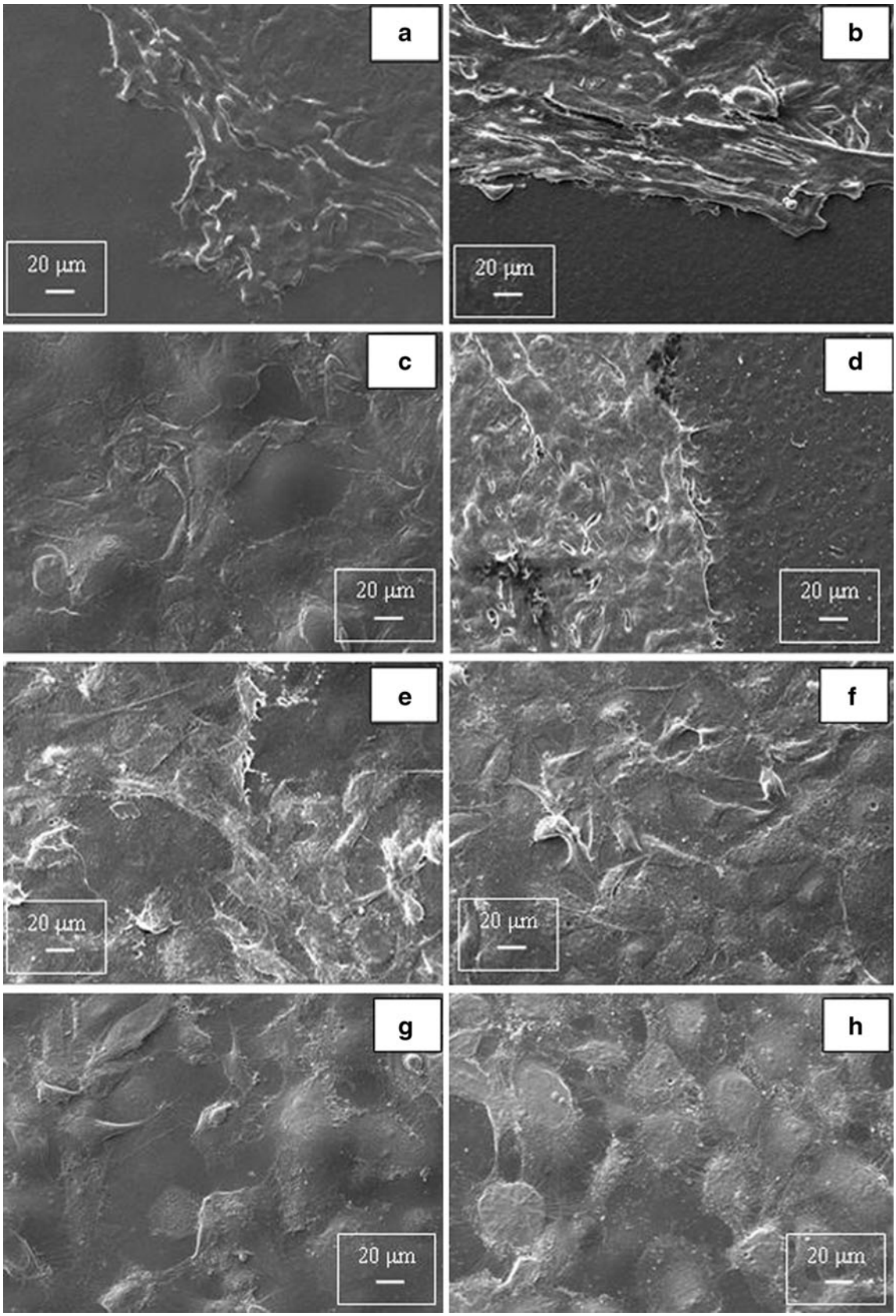

Fig. 9. SEM micrographs of cell-seeded PLLA/PCL films with different compositions (×1,000); at the end of 7 days of incubation: **a** 95/5, **b** 90/10, **c** 85/15, **d** 80/20; at the end of 14 days of incubation: **e** 95/5, **f** 90/10, **g** 85/15, **h** 80/20

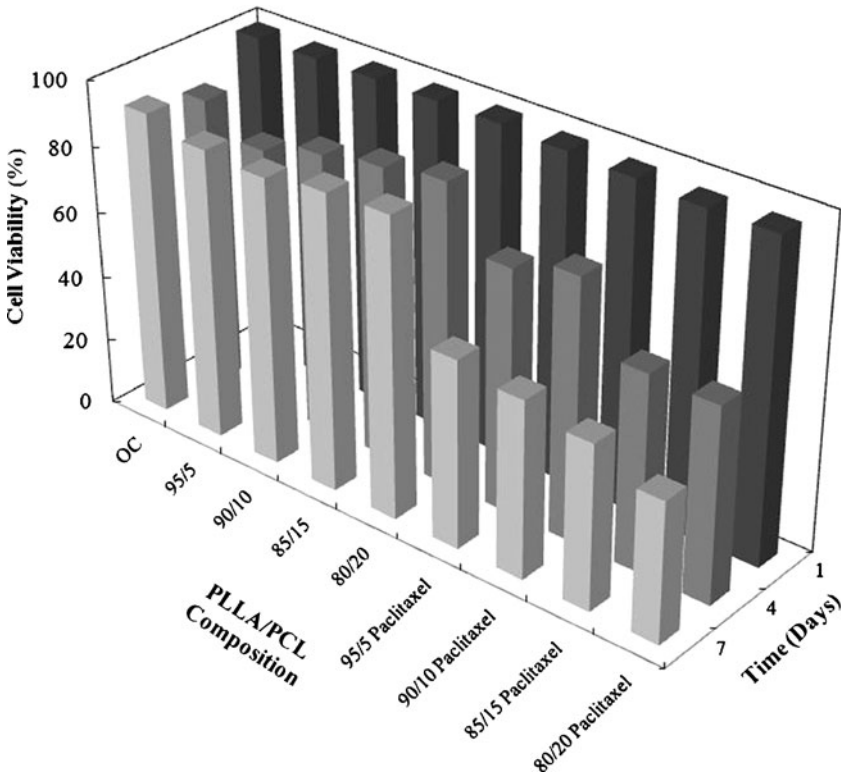

Fig. 10. Cell viability percentages of HUVECs due to paclitaxel elution from PLLA/PCL polymeric films at the days 1, 4, and 7

viability was observed compared to its control due to paclitaxel release at the end of day 7. Meanwhile, 35%, 38%, and 44% decreases were determined in cell viability in 95/5, 90/10, and 85/15 PLLA/PCL paclitaxel-loaded blends, respectively. Degradation accelerated by increasing PCL contents in the blend as shown in degradation studies should lead to a higher paclitaxel release which may cause the higher cell death observed. The results of this study proved that efficient drug release can be supplied by using the PLLA/PCL blend.

Axel *et al.* (25) investigated the effect of paclitaxel on monocultures of SMCs and co-cultures with human arterial endothelial cells. Nonstop paclitaxel exposure resulted in a complete and prolonged inhibition of the cells up to 14 days. In a subsequent *in vivo* study, local paclitaxel delivery to the carotid arteries of rabbits after induction of an atherosclerotic plaque caused significant decrease in the extent of the stenosis. However, there were no supplementary information about the inflammatory reactions and late endothelization risks.

Some studies have shown that some synthetic polymers, biodegradable or non-biodegradable, resulted in an important inflammatory and proliferative tissue response (26,27). However, our results showed that polymeric blends of PLLA/PCL were biocompatible. According to MTS data and SEM analysis, strong cellular attachment was observed and cell number on polymeric films increased by time.

Drug Release Studies

Initial drug release studies were performed embedding 7 mg paclitaxel in a 0.1-g polymer film with a thickness of 0.5–0.6 μm. All drug was released in about 25 days. As can be seen from Fig. 11, there is no significant difference in the release profiles of 95/10 and 85/15 PLLA/PCL mixtures. When drug release profiles were investigated from PLLA only, an abrupt drug release within 6 days was obtained possibly related to fast degradation rate of PLLA due to its highly amorphous structure.

In the following study, drug release studies were performed embedding 21 mg paclitaxel in a 0.3-g polymer film with a thickness of 0.16–0.18 μm. These studies have shown that all the drug was released in about 80 days as shown in Fig. 12.

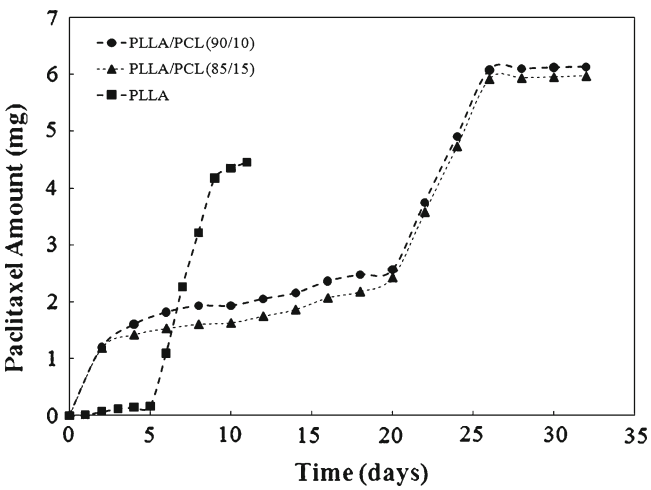

Fig. 11. Release of 7 mg paclitaxel from 0.1 g 90/10 and 85/15 PLLA/PCL polymer blends and PLLA polymer alone

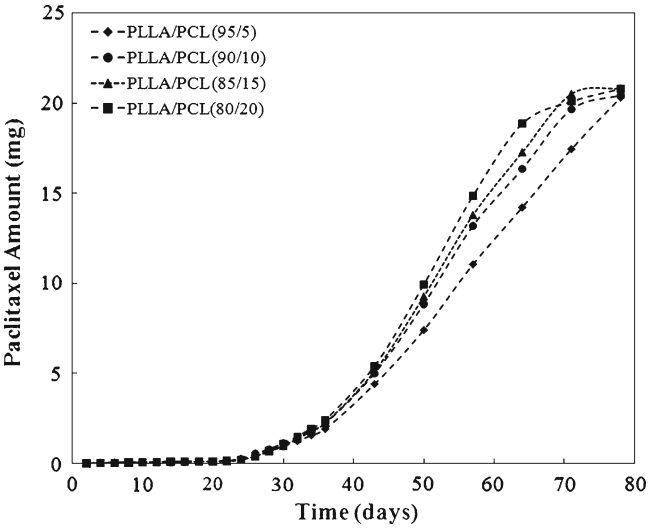

Fig. 12. Release of 21 mg paclitaxel from 0.3 g 95/5, 90/10, 85/15 and 80/20 PLLA/PCL polymer blends

Although the drug/polymer ratio is the same, the film thicknesses are about three times more in the case of high polymer content. The total amount of drug is released in 80 days *versus* 25 in the case of a film three times thicker. The release can take place by two mechanisms: degradation controlled or diffusion controlled. The surface area of the films increase as the thickness increases by about 3%, which would promote degradation and slightly increase the degradation rate. On the other hand, diffusion through the polymer is highly hindered as the thickness increases. Our release profiles clearly suggest a diffusion-controlled drug release.

This release has two stages where an initial slow release is followed by a fast release. In this initial stage of approximately 40 days, only about 10% of the drug is released into the medium. As the drug is likely to be embedded in the polymer matrix as suggested by SEM images of polymer blends containing paclitaxel, in the slow release region, the release of the drug is more likely to be degradation-controlled leading to a release exactly corresponding to the degradation profile. Assuming all drug to be evenly distributed in the polymer matrix and that it gets released with the degraded polymer, a drug release dataset is generated using the weight loss data. Figure 13 compares the actual drug release profile with the generated release profile for PLLA/PCL (90/10) blend. This figure clearly shows the initial stage of drug release to be degradation-controlled. The latter stage of the drug release should be dominated by the diffusion of the drug out of the polymer matrix into the medium which gives a faster release of the drug as seen in Figs. 12 and 13.

CONCLUSIONS

In this study, we have studied the PLLA/PCL blends with varying PLLA contents from 95 to 80 wt.% in terms of polymer properties such as crystallinity, porosity, and degradation, along with their biocompatibility and drug release profiles. The aim was to investigate the suitability of these blends for biomedical applications where the material should be biocompatible, biodegradable, and has the ability to incorporate and release a drug of interest. In this work, we have shown that without the complications of copolymerization or grafting, biocompatible polymers can be simply blended to tailor their crystallinity, degradation, and drug release profile. This gives rise to the possibility of other polymer blends to be tailored for more specific uses where a particular crystallinity, biodegradability, and drug release profile is desired.

The DSC analysis of the PLLA/PCL blend solution cast films showed that these blends are all phase-separated. The DSC data also indicated that %PLLA crystallinity increases with increasing PCL content (up to 15 wt.%) that there is a certain amount of interaction between PCL and PLLA at least in the presence of solvent and that the phase separation occurs during the solvent evaporation process. The decrease in PLLA crystallinity above 15 wt.% PCL content showed that above this content, the PCL disturbs the formation of ordered (crystalline) structure of PLLA chains.

The SEM analysis of the PLLA/PCL solution cast films etched with THF revealed that PCL is dispersed homogenously in the PLLA matrix as spherical domains and that the pore diameters of the PCL domains significantly increase as the PCL content increases. PLLA and PCL demix from each other during solvent evaporation and coalesce in separate domains to reduce the interfacial area and the amount of coalescense increases as the dispersed phase concentration increases. The SEM analysis of the surfaces of the solution cast films showed a porous morphology due to solvent evaporation process. These pores were larger than those of the PCL domain pore sizes which indicated that the solvent evaporation does not occur through the PCL domains.

These polymer blends degrade in physiological conditions, simulated by PBS buffer at pH 7.4. It was found that increasing PCL ratio in the blend, increases the degradation rate, although blending in general retards the overall degradation process. Although increasing PCL contents increases degradation rate, the degradation profiles of the blends follow the PCL degradation profile rather than PLLA which degrades at a higher rate than PCL. Thus, degradation

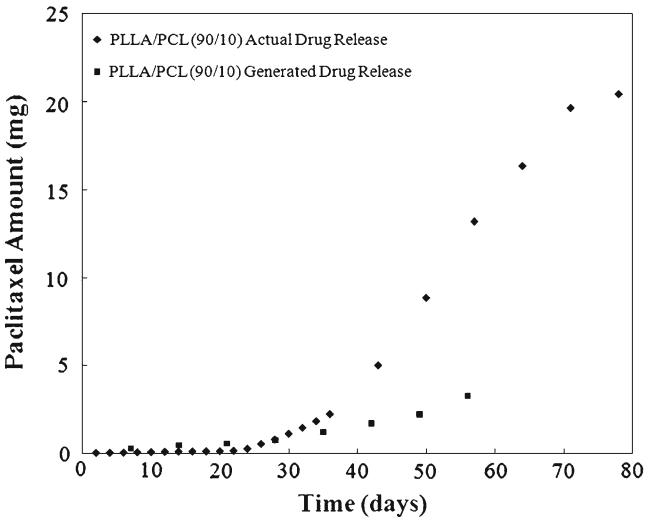

Fig. 13. Comparison of actual paclitaxel release from PLLA/PCL (90/10) polymer blend with generated release profile taking into account the weight loss of the blend

of PLLA is retarded upon addition of PCL possibly due to PCL being hydrophobic, acts like a surface-active agent, resides on the surface and makes it more challenging for water to penetrate into the amorphous regions of PLLA. As the PCL concentration gets higher, PCL surface to volume ratio reduces and degradation is relatively facilitated.

The biocompatibility of the polymer is the most significant criteria for its use as a biomaterial. The biocompatibility of the PLLA/PCL blends was proven by cell proliferation on PLLA/PCL blends as determined by MTS assay and cell attachment on the blends was also shown by SEM analysis. Apoptotic effect of PLLA/PCL blends with and without paclitaxel was tested by caspase-3 apoptosis assay. The results of the apoptosis assay showed an increase in cell death throughout 7 days of incubation for all blends. Drug-loaded blends caused more cell death than those without through 7 days of incubation as expected and the cell death was increased with increasing PCL contents.

It was shown that these blends are very suitable to release paclitaxel and the release times can be tailored by changing the composition of the blend and the film thickness. Drug release is shown to take place predominantly via diffusion mechanism, as well as due to film degradation. The results proved that drug release and degradation of the polymeric blend accelerated by the increment of PCL ratio.

In summary, it can be concluded that PLLA/PCL solution cast blend polymer films can be used for biomedical applications due to their controlled biodegradability depending on the blend composition, biocompatibility, and ability to release an incorporated drug.

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