Mechanical properties and cytotoxicity of PLA/PCL films

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Abstract

Thermodynamically immiscible poly(lactic acid) (PLA) and poly(ϵ -caprolactone) (PCL) were blended and solution-cast by adding the 3% compatibilizer (tributyl citrate, TBC) of the PCL weight. In the PLA/PCL composition range of 99/1–95/5 wt%, mechanical properties of the PLA/PCL films with TBC were always superior to those of the films without TBC. The tensile strength of 42.9 ± 3.5 MPa and the elongation at break of $10.3 \pm 2.7\%$ were observed for the 93/7 PLA/PCL films without TBC, indicating that PCL addition is effective for strength and ductility. However, the tensile strength of 54.1 ± 3.4 MPa and the elongation at break of $8.8 \pm 1.8\%$ were found for the 95/5 PLA/PCL with TBC, indicating that the effect of co-addition of PCL and TBC on mechanical properties of the films is more pronounced. No cytotoxicity was observed for the PLA/PCL films regardless of TBC addition.

Keywords Poly(lactic acid) · Poly(ϵ -caprolactone) · Compatibilizer · Tensile strength · Cytotoxicity · Cell proliferation

1 Introduction

The biodegradable polymers have been focused on as the candidate material of choice for environmental and biomedical applications, because of their excellent mechanical properties and biodegradability [1–8]. It is desirable to develop the renewable and biodegradable environmental materials to solve the problem caused by rapidly increasing disposable packaging materials designed for food and drugs [1]. The surgical sutures are also emerging as the temporary scaffolds requiring fast cell growth and tissue regeneration [3]. Sutures should be strong enough to hold tissue securely but flexible enough to be knotted. The biodegradable polymers possessing high strength, low modulus and good ductility are required for

the advanced environmental and biomedical applications [1–8].

Poly(lactic acid) (PLA) is a biodegradable aliphatic polyester from renewable resources, with the monomer produced by fermentation of polysaccharides [1]. Although PLA exhibits a very good balance in low deformation properties, its brittleness, low thermal resistance and limited gas barrier properties limit the wide-spread use [2]. On the contrary, poly(ϵ -caprolactone) (PCL) is a ductile polymer with high impact strength, but very low yield strength values and elastic modulus [1–4, 7]. Cohn and Salomon [3] reported that thrombosis and anastomotic intimal hyperplasia caused by the mechanical mismatches existing between the stiff vascular grafts and the host arteries can be avoided by using the PCL/PLA elastomer. Blending polymers rather than chemical modifications or synthesis of tailored macromolecules is one of the methods for modification of biodegradable polymers by improving thermal resistance and mechanical strength, leading to the well-balanced properties [1–6].

PLA and PCL are known to be thermodynamically immiscible [1–5]. Many researches have been studied to enhance the miscibility between PLA and PCL by using the triblock PLA–PCL–PLC copolyesters or the di-block PLA–PCL copolyesters as a compatibilizer [1, 4]. Small molecule compatibilizers of glycidyl methacrylate (GMA), dicumyl peroxide (DCP), tributyl citrate (TBC), triphenyl

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phosphite (TPP) and lysine triisocyanate (LTI) were also used to reduce the size of the dispersed phase and improve the toughness of the PLA matrix for the PLA/PCL blends. The addition of small amounts of the compatibilizers during the processing of the PLA/PCL blends helped to improve PLA/PCL ductility and impact strength by causing crosslinking reactions between PLA and PCL [1, 2, 4].

Sun et al. [1] have studied the effect of PCL and TBC on the mechanical and barrier properties of PLA/PCL films, however, their scope is limited to environmental packaging materials. Many researches have been mainly focused on synthesis and characterization of mechanical properties of PLA/PCL films [1–4]. In the present study, TBC was used as the compatibilizer of PLA and PCL. The biodegradable PLA/PCL films in the PLA/PCL concentration range of 99/1–93/7 wt% were synthesized by a solvent casting method to investigate the suitability of the films as tissue engineering applications by evaluating the mechanical properties and the biocompatibility.

2 Experimental

2.1 Materials

A commercial PLA (Ingeo Biopolymer 4044D) was supplied by NatureWorks (USA). PCL ($M_w = 80,000$ g/mol) purchased from Perstorp (Capa 6800, Sweden) was used to form the PLA/PCL blends. Dichloromethane (CH_2Cl_2 , Sigma–Aldrich, USA) with a density of 1.325 g/mL and molecular weight of 84.93 g/mol was purchased. TBC ($\text{C}_{18}\text{H}_{32}\text{O}_7$, Aldrich, UK) with a density of 1.043 g/mL and molecular weight of 360.44 g/mol was used as a compatibilizer. All chemicals were used as received without any further purification.

2.2 Preparation of PLA/PCL films

Prior to the blending, the PLA and PCL pellets were dried in a vacuum oven for 12 h at 95 and at 50 °C, respectively. A PLA/PCL solution dissolved in DCM was prepared by stirring (400 rpm) for 3 h at room temperature. The ratios of PLA/PCL blends were 99/1, 97/3, 95/5, and 93/7 wt%. The PLA/PCL blends were dissolved in DCM in the proportion of 1/10 in weight. The PLA/PCL films with the same PCL content were prepared by the addition of 3% TBC of the PCL weight as compatibilizer under the same conditions. The PLA/PCL solution was poured into the 80×60 mm² polytetrafluoroethylene molds, followed by drying for 24 h in air and in vacuum, respectively. The films with a diameter of 0.25 mm were peeled from the mold after drying. The same procedure was used for pure

PLA and PCL to compare the pure materials with the PLA/PCL hybrid films.

2.3 Characterization

The mechanical properties of PLA/PCL blends were investigated at room temperature using an Instron 5564 with a 2000 N force cell at a loading rate of 50 mm/min. The samples were prepared according to ASTM D-638 (type V) in the form of a dumbbell-shape [6]. The thickness of the samples was determined by measuring the samples immersed in liquid nitrogen using a dial gage. Dynamic mechanical tests were performed using Pyris Diamond dynamic mechanical analysis (DMA, Perkin Elmer, UK) with a Seiko Instruments Inc. analysis program [9]. Differential scanning calorimetry (DSC) and thermogravimetric (TG, Netzsch STAS 409C/31F, Germany) studies were also performed as the temperature rose from room temperature to 90 °C at a heating rate of 10 °C/min. The storage modulus (G') was measured at a fixed frequency of 1 Hz [1, 9]. $\tan \delta$, the glass transition temperature, was also examined. All experiments were performed in quintuplicate. Values in the text were expressed as the mean $\pm$ standard deviation, and $p < 0.05$ was considered statistically significant. The crystalline phase of the films was evaluated by using an X-ray diffraction (XRD) (MacScience, KFX-987228-SE, Japan) using the Cu $K\alpha$ radiation at 40 kV and 30 mA within the scan angles of 8°–50°.

2.4 Cytotoxicity

The extract test method was conducted on the PLA/PCL films to evaluate the potential of cytotoxicity on the base of the International Organization for Standardization (ISO 10993-5) [6–8, 10–13]. The PLA/PCL films were extracted aseptically in single strength Minimum Essential Medium [1X MEM, Dulbecco's Modified Eagles's Medium (Gibco) with 10% fetal bovine serum (Gibco) and 1% penicillin–streptomycin] with serum. The ratio of the PCL/PLA films to extraction vehicle was 1.25 cm²/mL. The 96-well plate was incubated at a temperature of 37 °C in a 5% CO₂ atmosphere. The test extracts were maintained in an incubator for 24 h. The test extracts were placed onto three separate confluent monolayers of L-929 (NCTC Clone 929, ATCC, USA) mouse fibroblast cells propagated in 5% CO₂. For this test, confluent monolayer cells were trypsinized and seeded in 10 cm² wells (35 mm dishes) using a micropipette. Simultaneously, triplicates of reagent control, negative control (high-density polyethylene film, RM-C), and positive control (polyurethane film, RM-A) were placed onto the confluent L-929 monolayers. All monolayers were incubated for 48 h at 37 °C in the presence of

5% CO₂. After incubation, the morphological change of the cell was examined to assess the biological reaction by using the inverted microscope (TS100-F, Nikon, Japan) and the iMark microplate absorbance spectrophotometer (Bio-Rad, USA) [6–8, 10]. The absorbance of the colored solution is quantified by measuring at a wavelength of 415 nm with the microplate absorbance spectrophotometer [6].

3 Results and discussion

XRD patterns of various PLA/PCL hybrid polymers are shown in Fig. 1, indicating that PCL is highly crystalline, while PLA is amorphous or slightly crystalline. A weak characteristic PLA crystalline peak located at $2\theta = 16.8^\circ$, superimposed on a broad amorphous peak background, was visible as PCL was added to PLA, implying that the PLA was composed of most amorphous and small amounts of crystalline. The crystalline peaks of PCL were located at $2\theta = 21.4^\circ$, 23.7° , 26.5° , and 29.3° . The XRD peaks of PLA/PCL polymers retained the characteristic PCL crystalline peaks at $2\theta = 26.5^\circ$ and 29.3° , superimposed on a broad PLA peak background with a peak at $2\theta = 16.8^\circ$. The absence of PCL peaks at $2\theta = 21.4^\circ$ and 23.7° was probably due to the overwhelming effect of amorphous PLA over PCL by hindering the mobility of the amorphous segments close to crystalline structure [2]. The PCL diffraction peaks at $2\theta = 26.5^\circ$ and 29.3° were shifted slightly towards higher angles. Sun et al. [1] reported that the addition of small amounts of PCL has a heterogeneous nucleation effect on the PLA crystallization, while the effect is weakened as the PCL content increases. This result is particularly important because it implies that the PLA/PCL blending effect can be controlled with the PCL

content. However, no appreciable variation in XRD peaks was detected.

DSC and TG were examined to investigate the state of the 95/5 PLA/PCL blend as a function of TBC concentration, as shown in Fig. 2. The melting temperature (T_m) of the 95/5 PLA/PCL scaffolds containing 1 wt% TBC was 65.5°C . It can be interpreted by the crystalline behaviors of PCL because T_m of 15 wt% PCL was 65.8°C [6]. T_m of the PLA/PCL hybrid scaffolds decreased from 65.5°C to 62.0°C with increasing the TBC content from 1 to 4% probably due to the blending effect of amorphous PLA. However, T_m increased dramatically to 67.7°C when TBC was added to the films more than 5%, suggesting that the miscibility effect of the PLA/PCL hybrid films was severely attenuated. In the present study, the 3 wt% TBC was selected to maintain the PLA contribution to the PLA/PCL hybrid scaffolds.

The temperature peaks of the $\tan \delta$ curves indicate T_g of the material and suggest whether two polymers are miscible. The T_g of the pure PLA film was reported to be 67 and 67.9°C , respectively [1, 5]. For PCL films, the T_g was -19.4 or -50°C [1, 5]. Our results revealed that the T_g values of the PLA/PCL films without TBC at higher temperatures decreased slightly from 50.3 to 48.8°C with increasing the PCL content, as shown in Fig. 3a. The T_g of the PLA/PCL films with TBC fluctuated between 44.2 and 50.6°C as PCL content increased (Fig. 3b). No particular trend of variation in T_g of the PCL/PLA films corresponding to lower temperatures was detected regardless of whether TBC is present. It was reported that the T_g values of PLA/PCL films containing TBC increased dramatically from -21.3 to -12.4°C as the PCL content rose from 1 to 5 wt%, implying that PLA and PCL were partially miscible due to the addition of TBC [1]. However, no appreciable variation in T_g was monitored in the present study. The improved miscibility of PLA and PCL may intensify the promotion effect of PCL on crystallization of

Fig. 1 XRD patterns of PCL and various PLA/PCL films

Fig. 2 DSC/TG data of various PLA/PCL films containing TBC

Fig. 3 Tan δ spectra of various PLA/PCL films **a** without and **b** with TBC

PLA as a sacrifice of transparency. To further evaluate the effect of PCL and TBC on the dynamic mechanical properties of PLA, the temperature dependence of G' on PLA and PLA/PCL films was investigated, as displayed in Fig. 4. The G' values of the films increased or decreased as a function of PCL content below and around T_g . The G' value of the PLA/PCL films regardless of the presence of TBC started to decrease with increasing the temperature from 33 to 50 °C. Although the 93/7 PLA/PCL films without TBC and the 95/5 PLA/PCL films with TBC have the highest G' at temperatures from 33 °C to T_g as shown in Fig. 4, any particular trend was not detected. Nevertheless, it is conceivable that the PCL addition is attributed to enhanced elastic properties of the PLA matrix as a result of the increased rigidity of the molecular chains [1, 6, 7].

The tensile strength and the elongation at break of the 10 wt% PLA scaffolds are 33.4 ± 2.5 MPa and $3.5 \pm 0.3\%$, respectively. Values in the text were expressed as the mean $\pm$ standard deviation, and $p < 0.05$ was considered statistically significant. Those of the 10 wt% PCL films are 11 ± 1.3 MPa and $780 \pm 180\%$, respectively, as shown in Figs. 5a and b. The elongation of the PCL film is 780%, which is beyond the scale range and is

Fig. 4 Storage modulus (G') of PLA/PCL films with various compositions under different temperatures

Fig. 5 **a** Tensile strength and **b** elongation at break of PLA, PCL and various PLA/PCL films

not shown fully on the graph (Fig. 5b). The tensile strength and the elongation at break of the PLA/PCL hybrid films without TBC increased slightly from 39.2 ± 2.3 to 42.9 ± 3.5 MPa and from 7.2 ± 1.0 to $10.3 \pm 2.3\%$ with increasing the PCL content from 1 to 7 wt%, as shown in Fig. 5. The highest tensile strength and ductility of the PLA/PCL hybrid films without TBC were observed for the 93/7 PLA/PCL films, which is in good agreement with the

Fig. 6 Photographs of cell morphologies: **a** 99/1, **b** 97/3, **c** 95/5, **d** 93/7 PLA/PCL films without TBC, **e** 99/1, **f** 97/3, **g** 95/5, and **h** 93/7 PLA/PCL films with TBC from WST assay (EZ-cytox) after exposing with the PLA/PCL suspensions for 48 h

results from DMA. The addition of PCL to PLA had the positive effect of promoting PLA matrix elasticity by increasing the rigidity of the molecular chains [1–4]. However, the strength of the PLA/PCL films with TBC exhibited different behavior. The tensile strength increased initially from 52.5 ± 7.8 to 54.1 ± 3.4 MPa as PCL content rose from 1 to 3 wt%, however, it started to decrease dramatically with further increasing PCL content, as depicted in Fig. 5a. The tensile strength of the 97/3 PLA/

PCL film with TBC (54.1 ± 3.4 MPa) were much higher than that (39.7 ± 1.97 MPa) of reference 1. In the present study, it is difficult to make precise comparison because of different test methods. However, no significant difference in the elongation at break among PLA/PCL films regardless of TBC addition was observed. The average elongation of PLA/PCL films were within the error range, as displayed in Fig. 5b. At the PLA/PCL composition range of 99/1–95/5 wt%, mechanical properties of PLA/PCL films with TBC

were always higher than those of PLA/PCL films without TBC probably due to the synergic contribution of PCL and TBC to PLA matrix. However, further addition of PCL was detrimental to the tensile strength of the PLA/PCL films due to the reduction in G' . In this study, the 97/3 PLA/PCL scaffolds with TBC was considered as the material of choice due to the tailored strength and ductility, which was much better than mechanical properties of pure PLA and PCL films.

A cytotoxicity test of the PLA/PCL films determines whether a product or compound will have a toxic effect on living cells [7, 8, 10–13]. The test extracts from the PLA/PCL films showed no evidence of causing cell lysis or toxicity regardless of TBC presence, as shown in Fig. 6. The PLA/PCL scaffolds, such as 99/1, 97/3, 95/5, 93/7 PLA/PCL films without TBC and with TBC, exhibited cell viability of 111, 109, 109, 111, 115, 112, 106 and 125% compared to the negative control, respectively, as measured at a wavelength of 415 nm by using the microplate absorbance spectrophotometer [6, 8]. All of the films examined in this study had excellent biocompatibility due to cell viability exceeding 100%. Therefore, it is conceivable that the PLA/PCL films exhibited no cytotoxicity under the condition of this study and are considered to be clinically safe and effective.

4 Conclusion

The PLA/PCL films were synthesized by using TBC for improving the mechanical properties of PLA and PCL without compensating the biodegradability. The highest tensile strength of 54.1 ± 3.4 MPa and the elongation at break of $8.87 \pm 1.84\%$ were obtained for the 97/3 PLA/PCL with TBC, implying that the strength of the PLA/PCL films was enhanced by the co-addition of PCL and TBC. As the PLA/PCL content was raised to 93/7 wt%, the elongation increased to $9.2 \pm 2.4\%$, but the strength decreased dramatically down to 37.2 ± 3.9 MPa. The PLA/PCL films regardless of TBC addition exhibited cell viability of more than 100%. Therefore, it is conceivable that the PLA/PCL films possessing tailored mechanical properties and excellent cytotoxicity are considered to be clinically safe and effective.

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Compliance with ethical standards

Conflict of interest The authors declares there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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