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Enzymatic Degradation and Porous Morphology of the Poly(L-lactide) and Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) Blends

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Abstract

Fully biodegradable polymer blends based on biosourced polymers, namely poly(L-lactide) (PLLA) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)) are prepared by melt compounding. The enzymatic degradation and porous morphology of PLLA/P(3HB-*co*-4HB) blends are investigated in detail. The lipase from *Pseudomonas mendocina* reveals preferred enzymatic degradation of P(3HB-*co*-4HB) but insignificant attack to PLLA in the blends. At the same time, proteinase K can degrade PLLA, but cannot degrade P(3HB-*co*-4HB). On account of the surface erosion mechanisms, the enzymatic degradation rates of both the P(3HB-*co*-4HB) and PLLA in the blends are improved because of the presence of the other component to increase the specific surface area. The results of the ^{1}H NMR and GPC indicated that there was no more intermediate products formed during the enzymatic degradation of the PLLA and P(3HB-*co*-4HB). Due to the specifity of the degradation enzymes, selective enzymatic degradation is adopted to degrade and remove one component from the blends, and various porous morphologies are acquired.

1. Introduction

The porous materials have received more and more attention in recent years due to their special surface property. They are widely used in gas-liquid separation, catalyst carrier, tissue engineering, and so on. There are many fabrication methods for the porous polymers, such as fiber bonding,[1] particulate leaching,[2] solvent casting and selective polymer extraction,[3] thermally induced phase separation (TIPS),[4] gas foaming, solid free-form (SFF),[5] etc. All approaches, however, have limitations. Such as particulate leaching allows for an easy control of pore structure while the using of organic solvent and the residual porogen may be the most disadvantages. Phase separation requires the dissolution of polymers in a solvent which will also be harmful to the cell and organs. Therefore, empoldering new methods for the fabrication of porous materials remain to be a challenge. Selective biodegradation was a newer method which was introduced to fabricate the porous polymers in recent years. Liu et al[6] fabricated poly(L-lactide) (PLLA)/poly(ε-caprolactone) (PCL) blend films by solution casting. The different biodegradable porous polymers can be acquired with selectively removing one of the phase by using the selective biodegradation of the proteinase K and *Pseudomonas* lipase to the PLLA and PCL, respectively. A similar method was adopted by Tsuji et al[7] and Hsiue et al[8] to prepare the porous films. However, all of the porous materials obtained through the selective biodegradation method were restricted to porous films, and the organic solvent was introduced inevitably, which was not environment-friendly.

PLLA, a promising aliphatic polyester derived from renewable resources, has been widely used in biomedical fields due to its biocompatibility, biodegradability, and good mechanical properties. The enzymatic degradation of the PLLA with different treatments was investigated in detail.[9-14] Tsuji et al revealed that the weight loss rates of the PLLA films after proteinase K-catalyzed hydrolysis increased with increasing the alkaline treatment time[9] or after hydrophilic polymer coating[10] due to the increased surface hydrophilicity. The

poly(3-hydoxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)), a member of the poly(hydroxyalkanoates) (PHAs) family which are another type of biosourced polymers, is desirable for applications in biomedical and environmental fields due to the good biodegradability, biocompatibility, relatively good toughness and process properties.[15-17] The biodegradation of P(3HB-*co*-4HB) was investigated extensively[18-23] and the results revealed that the degradation rate of P(3HB-*co*-4HB) depended strongly on its crystallinity and surface morphology due to its surface erosion mechanism. The biodegradation of PLA/P(3HB-*co*-4HB) blends were also investigated. The results revealed P(3HB-*co*-4HB) and PLA had different degradation rate due to different degradation mechanisms.[24] The addition of the P(3HB-*co*-4HB) in the PLA could accelerate the biodegradability of the blends whether in laboratory[25] or in soil condition.[24]

Up to now, it was confirmed that the lipase from *Pseudomonas mendocina* only degraded P(3HB-*co*-4HB) but did not attack PLA in the blends.[25] At the same time, proteinase K only degraded PLA, but not degraded P(3HB-*co*-4HB).[26, 27] Therefore, the selective biodegradation could be used to the blends of PLA/P(3HB-*co*-4HB) to produce the porous materials.

In this paper, different ratio of PLLA/P(3HB-*co*-4HB) blends were prepared by melt compounding. *Pseudomonas mendocina* lipase and proteinase K were used as specified degradation enzymes for P(3HB-*co*-4HB) and PLLA chains, respectively, and the effects of one component on the enzymatic degradation behavior of the other component were investigated in detail. Meanwhile, selective enzymatic degradation was adopted to degrade and remove one component from the blend, and porous morphology of PLLA/P(3HB-*co*-4HB) blends were investigated.

2. Experimental Section

2.1 Materials

The PLLA (Grade 4032D) used in this work was a commercially available product from Natureworks LLC (USA). It exhibited a weight-average molecular weight of 2.07×10^5 g mol^{-1} and polydispersity of 1.74 as determined by gel permeation chromatography (GPC). The P(3HB-*co*-4HB) was provided by Tianjin Guoyun Biotech (Tianjin, P. R. China). It exhibited a weight-average molecular weight of 4.97×10^5 g mol^{-1} and polydispersity of 1.85 (GPC analysis). The content of 4HB in the copolymer was 6.5 mol% determined by ^{1}H-nuclear magnetic resonance (NMR) spectroscopy.

2.2 Preparation of the Blends

Before processing, PLLA and P(3HB-*co*-4HB) were dried at 80 °C in a vacuum oven for 24 h. PLLA/P(3HB-*co*-4HB) blends with a series of weight ratios (100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90, 0/100)(wt/wt) were prepared by using a Haake batch internal mixer (Haake Rheomix 600, Karlsruhe, Germany) with a batch volume of 50 mL. The melt compounding was performed at 175 °C and a screw speed of 50 rpm during a total mixing time of 8 min, until the viscosity had reached a nearly constant value. After mixing, all the samples were cut into small pieces and then were hot-pressed at 180 °C for 3 min followed by cold-press at room temperature to form the sheets with thickness of 3 mm or 0.1 mm. The compression molding steps were carried out carefully in order to obtain the same treatment for every sample.

2.3 Enzymatic Degradation

The enzymatic degradation of the blend films for the P(3HB-*co*-4HB) was carried out in phosphate buffer (pH=8.0) containing *Pseudomonas mendocina* at 30 °C with shaking at 140 rpm. The composites films from the pressed sheets with thicknesses of 0.1 mm were chopped into square with gauge dimensions of 10 × 10 mm^2. Then all samples were placed in small

glass bottles containing the buffer and *Pseudomonas mendocina*. The samples were picked up after a fixed time interval, washed with distilled water, and dried to constant weight in a vacuum, and then the weights of the films were measured.

The enzymatic degradation of the blend films for PLLA was carried out in Tris-HCl buffered solution (pH=8.0) containing 0.2 mg mL^{-1} of proteinase K (Genview) at 45 ^{o}C with shaking at 140 rpm. Sample films (10×10×0.1 mm^3) were placed in small glass bottles filled with 1.5 mL Tris-HCl buffered solution containing proteinase K. The films were periodically removed, washed with distilled water, and dried to constant weight in a vacuum, and then the weights of the films were measured.

Selective enzymatic degradation was used to completely degrade and remove the P(3HB-*co*-4HB) or PLLA from the cryo-fractured surfaces of the blends to obtain the corresponding porous materials. The cryo-fractured surfaces were obtained through that the blends from the pressed sheets with thicknesses of 3 mm were immersed in liquid nitrogen for about 5 min, and then broken. The selective enzymatic degradation of the blends was carried out in the same conditions as above. When the P(3HB-*co*-4HB) or PLLA component in the sample was degraded completely, the sample was removed, washed with distilled water, and dried to constant weight in a vacuum.

2.4 Measurement and Observation

Thermal analysis was performed using a TA Instruments differential scanning calorimeter DSC Q20 (USA) under N_2 atmosphere. The specimens were crimp sealed in aluminum crucibles and had a nominal weight of about 4~6 mg. The specimens before and after enzymatic degradation were heated from the ambient temperature to 190 ^{o}C at a heating rate of 10 °C min^{-1} in the determination of the melting enthalpy (ΔH_m) of the blends. The degree of crystallinity of the P(3HB-*co*-4HB) was calculated by the following equations:

$$\chi_{c,\,P(3HB\text{-}co\text{-}4HB)}\,(\%) = \Delta H_{m,P(3HB\text{-}co\text{-}4HB)} \cdot 100 / [X_{P(3HB\text{-}co\text{-}4HB)} \cdot 146] \quad (1)$$

Where $X_{P(3HB\text{-}co\text{-}4HB)}$ is the weight ratio of P(3HB-*co*-4HB) in the blends and will be shown as follows. $\Delta H_{m,P(3HB\text{-}co\text{-}4HB)}$ (J g^{-1} of polymer) is the melting enthalpy of P(3HB-*co*-4HB), and 146 (J g^{-1} of P(3HB-*co*-4HB)) is the melting enthalpy of P(3HB-*co*-4HB) with 100% crystallinity reported by Barham P.J..[28]

Percentage weight loss was calculated according to the following equation using the weights of a film before and after degradation (W_{before} and W_{after} respectively)

$$\text{Non-normalized weight loss(wt\%)} = 100(W_{before} - W_{after})/W_{before} \quad (2)$$

The non-normalized weight loss obtained by Equation (2) was normalized by degradable PLLA or P(3HB-*co*-4HB) weight fraction using the equations:

$$\text{Normalized P(3HB-}co\text{-4HB) weight loss (wt\%)} = \text{Non-normalized weight loss}/X_{P(3HB\text{-}co\text{-}4HB)} \quad (3)$$

(for *Pseudomonas mendocina* lipase-catalyzed enzymatic degradation).

$$\text{Normalized PLLA weight loss (wt\%)} = \text{Non-normalized weight loss}/(1\text{-}X_{P(3HB\text{-}co\text{-}4HB)}) \quad (4)$$

(for proteinase K-catalyzed enzymatic degradation), and

$$X_{P(3HB\text{-}co\text{-}4HB)} = W_{P(3HB\text{-}co\text{-}4HB)}/(W_{PLLA} + W_{P(3HB\text{-}co\text{-}4HB)}) \quad (5)$$

Where $W_{P(3HB\text{-}co\text{-}4HB)}$ and W_{PLLA} are the weights of P(3HB-*co*-4HB) and PLLA, respectively, in a film. The experimental weight loss values represent averages of measurements from the three replicate specimens.

The ^{1}H NMR spectra were recorded using a Bruker 300 MHz spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard.

The weight-average molecular weight (M_w), number-average molecular weight (M_n) and molecular weight distribution were determined by Gel Permeation Chromatography (GPC) conducted in $CHCl_3$ at 35°C, at a flow rate of 1 mL min^{-1} using a Waters 515 HPLC pump

solvent delivery system with a set of two Waters Styragel HT4 and HT3 column and Waters 2414 refractive index detector. Polystyrene standards with narrow molar mass distribution were used to generate a calibration curve.

The cryo-fractured surfaces after degraded and removed the one component from the blends were coated with a thin layer of gold and then they were observed with a field emission scanning electron microscopy (XL30 ESEM FEG, FEI Co., Eindhoven, The Netherlands) at an accelerating voltage of 10 kV to obtain the porous morphology of the PLLA and P(3HB-*co*-4HB), respectively. The size of the pore for the porous polymers was acquired with the Image Analysis.

3. Results and Discussions

3.1 Weight Loss of PLLA/P(3HB-*co*-4HB) Blends after Enzymatic Degradation

3.1.1 *Pseudomonas Mendocina* Lipase-Catalyzed Degradation of P(3HB-*co*-4HB) Component

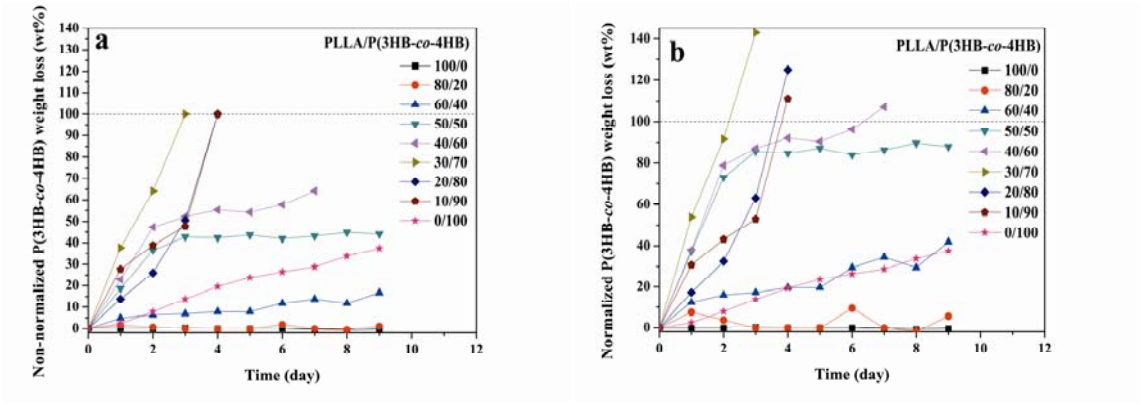


Figure 1 The non-normalized (a) and normalized (b) P(3HB-*co*-4HB) weight loss profiles of PLLA/P(3HB-*co*-4HB) blend films as a function of time during the *Pseudomonas mendocina* lipase-catalyzed degradation.

Figure 1 shows the non-normalized and normalized weight loss profiles of the films as a result of *Pseudomonas mendocina* lipase-catalyzed degradation. The rate of enzymatic degradation could be determined from the slope of the weight loss against time. Here, only the P(3HB-*co*-4HB) in the films was degraded since the neat PLLA had little weight loss. As is shown in Figure 1, the weight loss of the PLLA/P(3HB-*co*-4HB) blend was increased as a function of time during the enzymatic degradation. Neat P(3HB-*co*-4HB) showed a gently curve which indicated a slow enzymatic degradation rate. However, the blend with the addition of 10 wt% PLLA into the P(3HB-*co*-4HB) exhibited greatly increasing of the enzymatic degradation rate. The samples with PLLA content of 30 wt%, 40 wt%, 50 wt% had the most fast enzymatic degradation rate and these samples could be completely biodegraded within four days. A lot of evidence demonstrated that the rate of enzymatic hydrolysis of biodegradable materials was dependent on the degree of crystallinity[18, 21, 22] and the surface area of the polymer[18, 19] exposed to enzymatic hydrolysis. In order to clarify the reason of the increased enzymatic degradation rate for the blends, the thermal properties of the blends were first investigated to determine the degree of crystallinity of the P(3HB-*co*-4HB) in the blends.

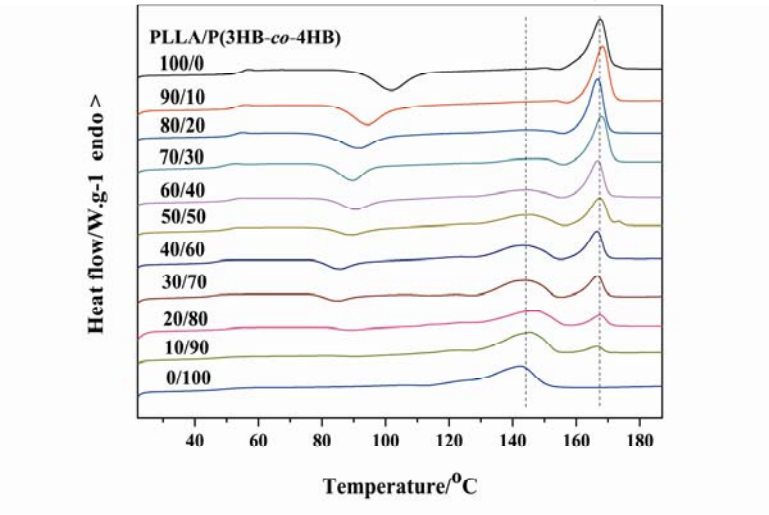


Figure 2 The DSC thermograms of the first heating traces for PLLA/P(3HB-*co*-4HB) blends with different compositions.

Figure 2 shows the DSC thermograms of the first heating traces for PLLA/P(3HB-*co*-4HB) blends with different compositions. The melting enthalpy (ΔH_m) of the P(3HB-*co*-4HB) derived from the DSC curves and the corresponding calculated degree of crystallinity of the P(3HB-*co*-4HB) are summarized in **Table 1**. In Figure 2, the neat P(3HB-*co*-4HB) and neat PLLA exhibited only one melting temperature (T_m) of 142.9 ^oC and 167.5 ^oC, respectively. While for the blends of PLLA/P(3HB-*co*-4HB) with various compositions, there were two melting temperatures corresponding to the P(3HB-*co*-4HB) and PLLA, respectively, and the melting enthalpy of the two components varied with the content of the component.

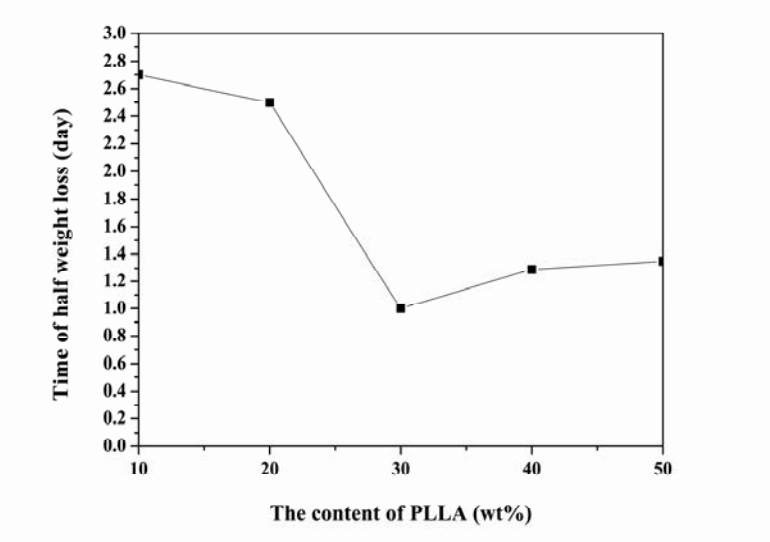
Table 1 The parameters of the PLLA/P(3HB-*co*-4HB) blends

PLLA/ P(3HB-*co*-4HB)	$\Delta H_{m,P(3HB\text{-}co\text{-}4HB)}$[a)] ($J\ g^{-1}$)	$\chi_{c,P(3HB\text{-}co\text{-}4HB)}$ (%)	Size of porous PLLA (μm)	Size of porous P(3HB-*co*-4HB) (μm)
100/0	-	-	-	-
90/10	41.04	28.1	0.39±0.15	-
80/20	40.13	27.5	1.08±0.39	-
70/30	43.07	29.5	2.65±1.76	-
60/40	42.53	29.0	13.15±6.41	-
50/50	41.24	28.2	-	-
40/60	42.83	29.3	-	15.91±8.29
30/70	40.87	28.0	-	3.14±4.40
20/80	41.20	28.2	-	1.73±0.70
10/90	42.11	28.8	-	0.61±0.23
0/100	41.14	28.2	-	-

[a)] $\Delta H_{m,P(3HB\text{-}co\text{-}4HB)}$ are corrected for the content of P(3HB-*co*-4HB) in the blends.

From Table 1, it was clear that the addition of PLLA showed little effect on the variation of the degree of crystallinity of P(3HB-*co*-4HB) component, indicating that the enzymatic degradation result of the P(3HB-*co*-4HB) was not attributed to the change of the crystallization. On the other hand, enzymatic hydrolysis started on the surface and at physical lesions on the polymer and proceeded to the inner part of the material.[22] The addition of the PLLA increased the specific surface area of the P(3HB-*co*-4HB). According to Han et al.,[25] the PLLA and P(3HB-*co*-4HB) were immiscible, therefore, the diffusion rate of the enzymatic

198 molecules into the interface between the two phase could be improved. Thus the
199 biodegradation rate of P(3HB-*co*-4HB) could be increased. The normalized weight loss of the
200 films with 10 wt%, 20 wt%, and 30 wt% content of PLLA increased to over 100 wt% for
201 degradation times longer than 4d. This may be explained by the release of PLLA into the
202 media with the removal of P(3HB-*co*-4HB) molecules. In other words, the dispersed PLLA
203 molecules contained in the continuous P(3HB-*co*-4HB)-rich phase may be liberated by the
204 enzymatic hydrolysis and removal of P(3HB-*co*-4HB). For the samples with content of 60
205 wt%, 80 wt% PLLA, the degradation rate was decreased to a very low value. This may be
206 ascribed to that with the increase of the PLLA content, the P(3HB-*co*-4HB) was trapped in the
207 continuous of PLLA domain, and the diffusion of the *Pseudomonas mendocina* lipase into the
208 interfaces of the two polymer phases was hindered, thus the P(3HB-*co*-4HB) could not get in
209 touch with the enzymatic molecules sufficiently.



210

211 **Figure 3** The time of half P(3HB-*co*-4HB) weight loss profiles of the PLLA/P(3HB-*co*-4HB)
212 blend films versus the content of the PLLA during the *Pseudomonas mendocina*
213 lipase-catalyzed degradation.

214 Time of half weight loss is an important parameter to determine and compare degradation

behaviors. **Figure 3** shows the time of half P(3HB-*co*-4HB) weight loss profiles of the films versus the content of the PLLA. It showed that the time of half weight loss of the P(3HB-*co*-4HB) decreased with increasing the content of the PLLA, indicating the accelerated enzymatic degradation of the P(3HB-*co*-4HB). While there was an optimum PLLA content of 30 wt% for the enzymatic degradation of P(3HB-*co*-4HB), after this peak, the enzymatic degradation of P(3HB-*co*-4HB) began to decline with increasing the content of the PLLA due to the wraparound effect of the PLLA.

3.1.2 Proteinase K-Catalyzed Degradation of PLLA Component

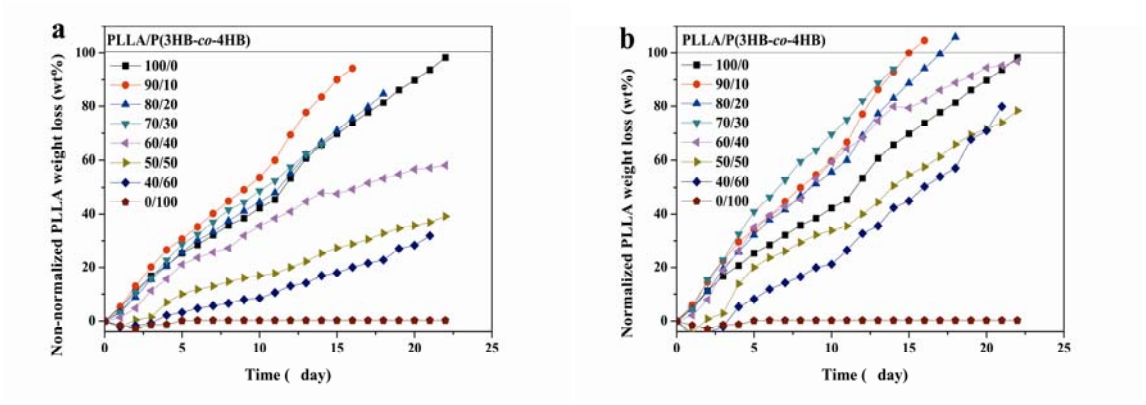


Figure 4 The non-normalized (a) and normalized (b) PLLA weight loss profiles of PLLA/P(3HB-*co*-4HB) blend films as a function of time during the proteinase K-catalyzed degradation.

Figure 4 shows the non-normalized and normalized weight loss profiles of PLLA/P(3HB-*co*-4HB) blend films as a function of time during the proteinase K-catalyzed degradation. The rate of enzymatic degradation could be determined from the slope of the weight loss against time. As evident from Figure 4, for all the samples, the weight losses of the films increased with time due to the enzymatic degradation of the PLLA except for the neat P(3HB-*co*-4HB) which almost had no weight loss. It confirmed that the proteinase K can

only degrade PLLA, but not degrade P(3HB-*co*-4HB). For the blends with 10 wt%, 20 wt%, 30 wt% content of P(3HB-*co*-4HB) that the rates of enzymatic degradation were fast even at the initial stage whether they were normalized or not. **Figure 5** shows the time of half PLLA weight loss profiles of the films versus the content of the P(3HB-*co*-4HB) during the proteinase K-catalyzed degradation.

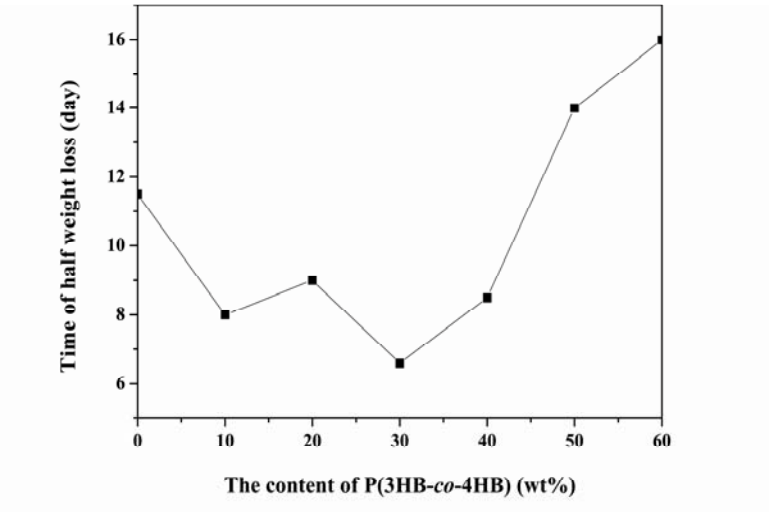


Figure 5 The time of half PLLA weight loss profiles of the PLLA/P(3HB-*co*-4HB) blends films versus the content of the P(3HB-*co*-4HB) during the proteinase K-catalyzed degradation.

As is shown in Figure 5, The time of half PLLA weight loss decreased with the addition of the P(3HB-*co*-4HB) when the P(3HB-*co*-4HB) content was lower than 40 wt%, indicating the addition of P(3HB-*co*-4HB) accelerated the degradation of PLLA by proteinase K. An optimum P(3HB-*co*-4HB) content for the enzymatic degradation of PLLA was 30 wt%. When the P(3HB-*co*-4HB) content was more than 40 wt%, the enzymatic degradation of PLLA was restricted. As mentioned above, the rate of enzymatic hydrolysis of biodegradable materials was dependent on the degree of crystallinity[18, 21, 22] and the surface area of the polymer[18, 19] exposed to enzymatic hydrolysis. From Figure 2, it should be noted that there was an obvious

cold crystallization peak for the PLLA in neat PLLA and its blends. In addition, the cold crystallization enthalpy was very similar with the melting enthalpy of the PLLA for each sample, which indicated that the PLLA was primarily amorphous and the enzymatic degradation result of the PLLA was not influenced by its crystallization. Since the degradation of PLLA was mainly stared with a hydrolysis, the water absorption was very important in the degradation process. The two-phase nature of the blends due to the immiscibility resulted in a higher water absorption and proteinase K diffusion from the surface to the inside of the blends than that of neat PLLA, thus led to the acceleration of PLLA hydrolysis there, thereby accelerating degradation.[7, 25, 29, 30] In other words, the degradable surface areas of PLLA domains per unit mass were increased by phase separation and particulate domain formation. A counter-example shows that the addition of poly[(L-lactide)-*co*-(ε-carprolactone)] as a compatibilizer between PLLA and PCL reduced ptoteinase K-catalyzed enzymatic degradation rate due to the decreased hydrolysable interfacial area between the PLLA-rich and PCL-rich phase.[31] That is to say that the decreasing voids and cavities between the phase interface bring down the proportion of the entrance of the proteinase K then reduce the enzymatic degradation rate of PLLA. The mechanism of the accelerated enzymatic degradation rate of PLLA is the same as that of P(3HB-*co*-4HB). When the content of P(3HB-*co*-4HB) increased to 50 wt% and 60 wt%, the rate of enzymatic degradation were decreased compared to the neat PLLA. This may be due to that the PLLA was contained by P(3HB-*co*-4HB)-rich phase and cannot get in touch with the enzymatic molecules sufficiently. The normalized weight loss of the films with 10 wt%, 20 wt% content of P(3HB-*co*-4HB) increased to over 100 wt% for hydrolysis times longer than 15d. This also can be explained by the release of P(3HB-*co*-4HB) into the hydrolysis media with the removal of PLLA molecules just as the enzymatic degradation of the P(3HB-*co*-4HB).

3.2 The Composition Changes of PLLA/P(3HB-*co*-4HB) Blends characterized by NMR

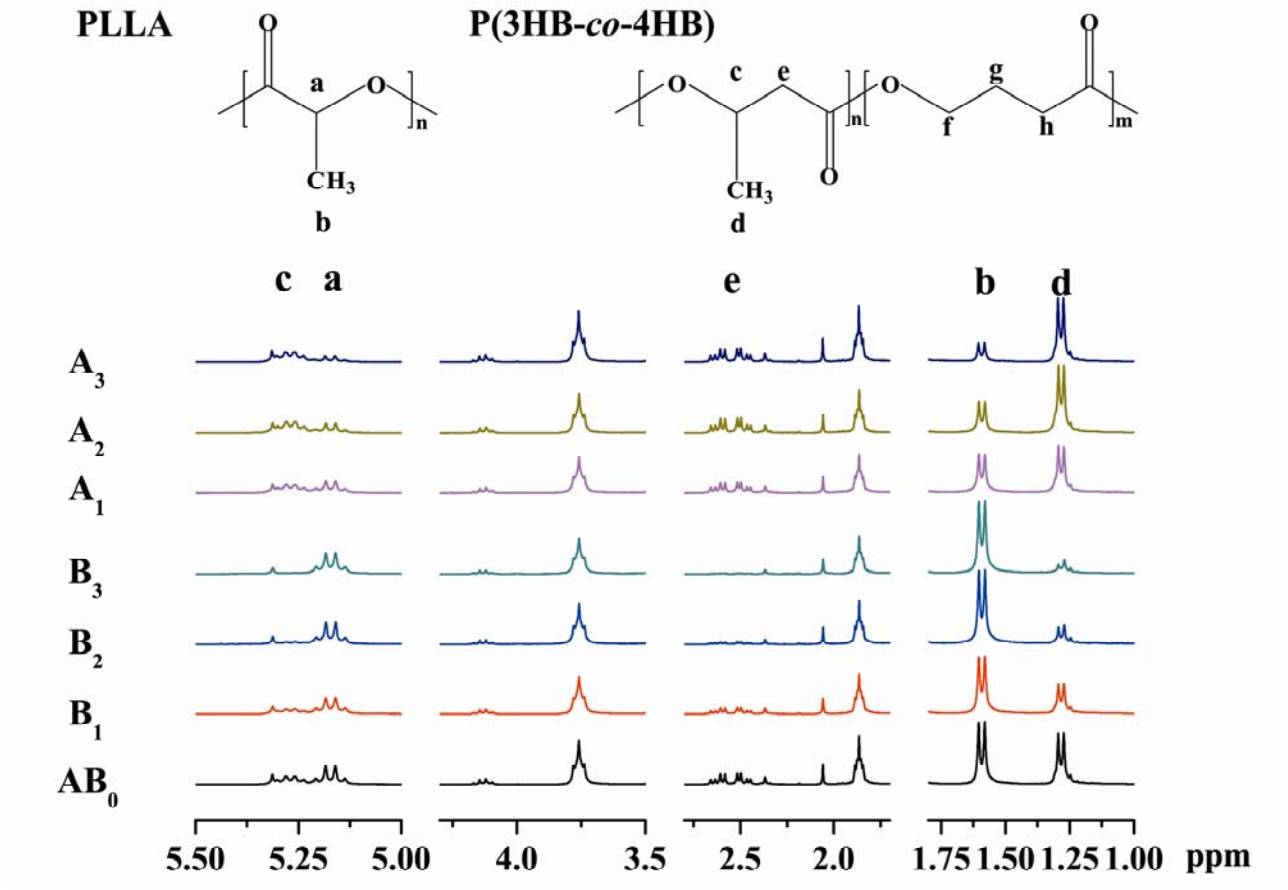


Figure 6 Selected regions of the ^{1}H NMR spectra of PLLA/P(3HB-*co*-4HB) blends films (AB_0) before and after *Pseudomonas mendocina* lipase catalyzed degradation of P(3HB-*co*-4HB) for (B_1) 1day; (B_2) 2day; (B_3) 9day and proteinase K catalyzed degradation of PLLA for (A_1) 5day; (A_2) 10day; (A_3) 22day.

The ^{1}H NMR experiment of the remaining PLLA/P(3HB-*co*-4HB) blend films with 50 wt% content of P(3HB-*co*-4HB) before and after specific times of degradation by *Pseudomonas mendocina* lipase and proteinase K, respectively, was carried out and **Figure 6** depicted the most informative regions of the spectra (which are most important for determination of chemical structure). In Figure 6, AB_0 shows the ^{1}H NMR spectrum of PLLA/P(3HB-*co*-4HB) blend films before enzymatic degradation, and the selected regions of ^{1}H NMR spectrum of

the investigated samples corresponding to the occurrence of signals ascribed to PLLA (δ = 5.16, 1.58 ppm) and P(3HB-*co*-4HB) (δ = 5.27, 2.51, 1.28 ppm). The integral area of the characteristic peaks was almost 1:1 at δ = 1.58 ppm and δ = 1.28 ppm corresponding to the methyl group protons of PLLA and P(3HB-*co*-4HB), respectively, indicating the composition ratio of PLLA and P(3HB-*co*-4HB) was 1:1. When the blend film was degraded by *Pseudomonas mendocina* lipase for 1 day, the intensities of the signals characteristic for the methyl, methylene, methine (δ = 1.28, 2.51, 5.27 ppm) of the P(3HB-*co*-4HB) were decreased while the characteristic peak corresponding to the PLLA (δ = 5.16, 1.58 ppm) remained unchanged. By comparing the integral area of the characteristic peaks between the δ = 1.58 ppm and δ = 1.28 ppm, the content of the P(3HB-*co*-4HB) in the remained films could be calculated as 36%, almost matching with the non-normalized weight loss of 18% for the blend films with the P(3HB-*co*-4HB) content of 50 wt% degraded for 1 day. When the degradation was proceeded for 9 day, the signals for P(3HB-*co*-4HB) were almost vanished from sight, implied that the P(3HB-*co*-4HB) could be removed efficiently from the PLLA. Correspondingly, after the proteinase K catalyzed degradation, the characteristic peaks corresponding to the PLLA (δ = 5.16, 1.58 ppm) decreased little by little while those of the P(3HB-*co*-4HB) (δ = 1.28, 2.51, 5.27 ppm) kept constant. At the end of the degradation, the signals of PLLA almost disappeared. The composition changes in the PLLA and P(3HB-*co*-4HB) could be matched well with the corresponding weight loss of the PLLA/P(3HB-*co*-4HB) films after enzymatic degradation.

It should be emphasized that there were no new signals appeared during the process of the enzymatic degradation either by the *Pseudomonas mendocina* lipase or by proteinase K compared to the ^{1}H NMR spectrum before enzymatic degradation, which indicated that there was no other form of materials fabricated. In other words, there were no intermediate products formed during the enzymatic degradation process for both the PLLA and P(3HB-*co*-4HB).

According to Albertsson et al.,[32] the finial degradation products of the pure PLLA catalyzed by proteinase K were water-soluble lactic acid and its oligomers. For the *Pseudomonas mendocina* lipase-catalyzed degradation of the P(3HB-*co*-4HB),[33] the degradation products were a mixture of oligomers and finally were metabolized by the *Pseudomonas mendocina* to CO_2 and H_2O. This may be attributed to the surface erosion mechanisms of the enzymatic degradation for both the PLLA and P(3HB-*co*-4HB). In short, only the surface of the samples was eroded, the macromolecular chains were cut into the corresponding soluble oligomers immediately while the internal remained almost unchanged during the enzymatic degradation process, which could also be confirmed by the results of the GPC (shown as follows).

3.3 Molecular Weight Change of PLLA/P(3HB-*co*-4HB) Blends before and after Enzymatic Degradation

Table 2. Molecular weights and dispersity index of PLLA/P(3HB-*co*-4HB) (50/50 wt/wt) before and after enzymatic degradation

PLLA/P(3HB-*co*-4HB) (50/50 wt/wt)		$M_n/10^5$ (g mol^{-1})	$M_w/10^5$ (g mol^{-1})	M_w/M_n
Before enzymatic degradation		0.61	1.18	1.95
After *Pseudomonas mendocina* lipase catalyzed degradation	1 day	0.87	1.32	1.52
	2 day	0.89	1.36	1.53
	9 day	0.97	1.47	1.51
After Proteinase K catalyzed degradation	5 day	0.74	1.21	1.64
	10 day	0.79	1.33	1.69
	22 day	0.71	1.13	1.58

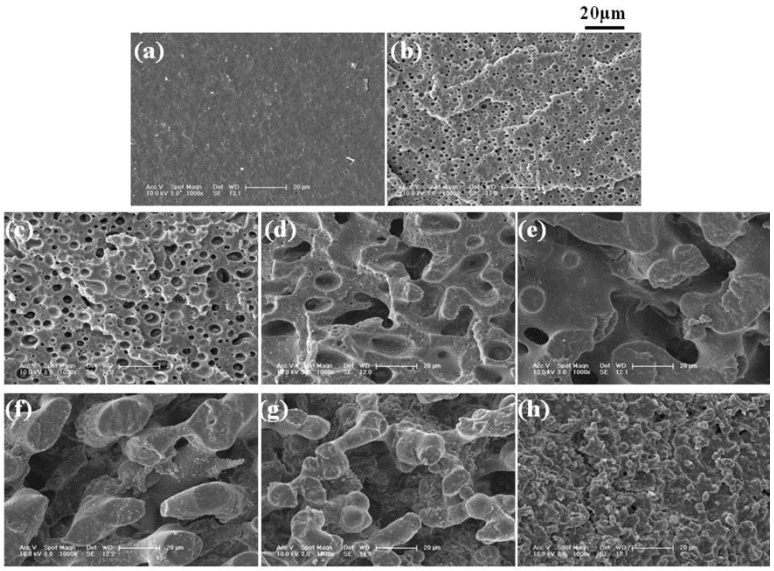
The GPC traces of the remaining PLLA/P(3HB-*co*-4HB) blend films with 50 wt% content of PLLA after specific times of degradation by *Pseudomonas mendocina* lipase and proteinase K, respectively, were performed and the detailed data of M_w, M_n and M_w/M_n before and after enzymatic degradation are summarized in **Table 2**. The results revealed that there was only one retention volume peak (not shown here) before enzymatic degradation for the PLLA/P(3HB-*co*-4HB) (50/50 wt/wt) blend films and the value of M_w was around 1.18×10^5 g mol^{-1} shown as Table 2. It should be noted that the results of GPC analyses presented in Table

2 were calculated for the blend which contained two components, PLLA and P(3HB-*co*-4HB). Thus in Table 2, the presented molecular mass and dispersity values were apparent. The only one retention volume peak may be due to that the PLLA and P(3HB-*co*-4HB) suffered from certain thermal degradation after the blending and molding process and the molecular weight of them became too close to distinct from each other. What's more, the PLLA/P(3HB-*co*-4HB) blend films showed almost the same molecular weight and molecular weight distributions before and after either the *Pseudomonas mendocina* lipase or proteinase K catalyzed degradation shown as Table 2. The M_w of all the PLLA/P(3HB-*co*-4HB) blend films remained almost unchanged after the enzymatic degradation either by the *Pseudomonas mendocina* lipase or proteinase K catalyzed, implying no accumulation of the low-molecular weight enzymatic degraded components. This was accordance with the results of the ^{1}H NMR and further confirmed the surface erosion mechanisms of the enzymatic degradation for both the PLLA and P(3HB-*co*-4HB). At the same time, the M_w/M_n list in Table 2 was 1.95 before enzymatic degradation and had a little decrease both after the *Pseudomonas mendocina* lipase and proteinase K catalyzed degradation, which may be due to that with the process of the degradation, one of the component was removed gradually and there was almost only one component after the enzymatic degradation eventually, therefore the molecular weight distribution became narrower.

3.4 Porous Morphology of PLLA/P(3HB-*co*-4HB) Blends after Degradation

The previous section investigated the influence of one component on the biodegradation properties of the other component and the results revealed that the enzymatic degradation process of the *Pseudomonas mendocina* lipase and the proteinase K for the blends with different ratio of PLLA/P(3HB-*co*-4HB) could be finished completely within 9 and 22 days, respectively. Therefore, selective enzymatic degradation for the biodegradation of the

356 P(3HB-*co*-4HB) was proceeded for 9 days, and the biodegradation of the PLLA was 22 days
357 to confirm the complete removal of the corresponding component thus to acquire the certain
358 porous structure.



359

360 **Figure 7** The porous PLLA with selectively removal of the P(3HB-*co*-4HB), the content of
361 the P(3HB-*co*-4HB): (a) 10 wt%; (b) 20 wt%; (c) 30 wt%; (d) 40 wt%; (e) 50 wt%; (f) 60
362 wt%; (g) 70 wt%; (h) 80 wt%.

363 **Figure 7** presents the porous PLLA with selectively removal of the P(3HB-*co*-4HB) by
364 *Pseudomonas mendocina* after selective enzymatic degradation for 9 days, and the contents of
365 the P(3HB-*co*-4HB) were (a) 10 wt%; (b) 20 wt%; (c) 30 wt%; (d) 40 wt%; (e) 50 wt%; (f) 60
366 wt%; (g) 70 wt%; (h) 80 wt%, respectively. The size of the porous PLLA obtained was listed
367 in Table 1. Because *Pseudomonas mendocina* can only degraded P(3HB-*co*-4HB), but not
368 degraded PLLA, it can be concluded that the black pores in the cryo-fractured surfaces of the
369 blends must be formed by the removal of P(3HB-*co*-4HB) and the pore morphology should
370 agree with that of P(3HB-*co*-4HB) in the initial blends. As is shown in Figure 7, when the
371 content of the P(3HB-*co*-4HB) was 10 wt%, the PLLA had a pore of 0.39 ± 0.15 μm and the
372 pore in the PLLA become more and more bigger with the increasing content of the

P(3HB-*co*-4HB). Interestingly, when the content of the P(3HB-*co*-4HB) increased to 40~60 wt%, there were interconnected pores appeared due to the co-continuous phase formed. These samples that have interconnected pores are biodegradable, biocompatible and innocuous, which will be appropriate for the application of tissue engineering. Further increasing the content of the P(3HB-*co*-4HB), the blends evolved into the sea-island structure, and the PLLA phase became the "island" of sea-island structure. When the content of the P(3HB-*co*-4HB) increased to 80 wt%, the remainder PLLA phase were particles therefore the sample could not keep its integrity.

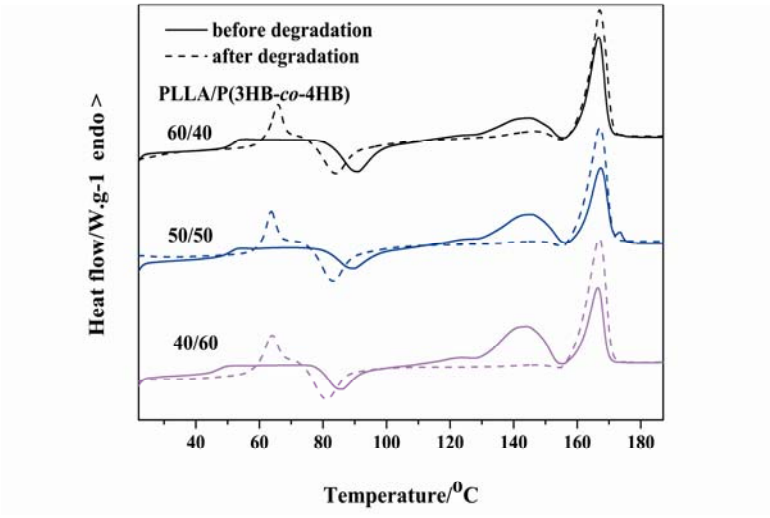


Figure 8 The DSC thermograms of the cryo-fractured surfaces of the PLLA/P(3HB-*co*-4HB) blends with the content of 40 wt%, 50 wt%, 60 wt% of the PLLA before and after *Pseudomonas mendocina* lipase-catalyzed degradation of P(3HB-*co*-4HB).

In order to confirm that the P(3HB-*co*-4HB) in the porous PLLA was removed completely, the differential scanning calorimeter (DSC) was used to study the thermal properties of the blends after enzymatic degradation. **Figure 8** shows the DSC thermograms of the cryo-fractured surfaces of the PLLA/P(3HB-*co*-4HB) blends with the content of 40 wt%, 50 wt%, 60 wt% of the PLLA before and after *Pseudomonas mendocina* lipase-catalyzed

degradation of P(3HB-*co*-4HB). It can be found that there was only the T_m peak of the PLLA for the blends containing 40 wt%, 50 wt%, 60 wt% PLLA after enzymatic degradation, this confirmed that the P(3HB-*co*-4HB) was removed completely for the samples by selective enzymatic degradation. It provided an accessible method for the fabrication of porous polymers through selective biodegradation.

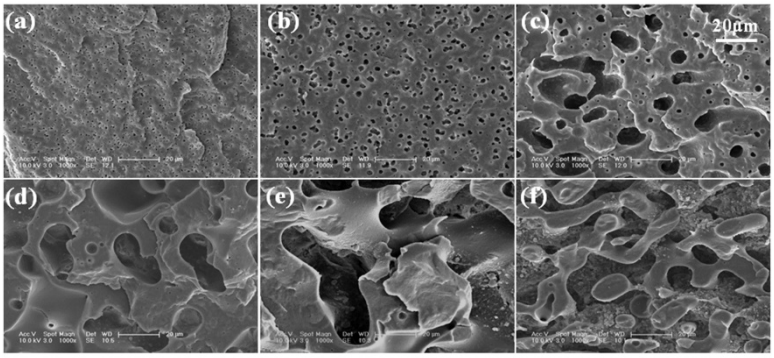


Figure 9 The porous P(3HB-*co*-4HB) with selectively removal of the PLLA, the content of the PLLA: (a) 10 wt%; (b) 20 wt%; (c) 30 wt%; (d) 40 wt%; (e) 50 wt%; (f) 60 wt%.

Figure 9 shows the corresponding porous P(3HB-*co*-4HB) with selectively removal of the PLLA, the contents of the PLLA were (a) 10 wt%; (b) 20 wt%; (c) 30 wt%; (d) 40 wt%; (e) 50 wt%; (f) 60 wt%;respectively. There was a similar phase inversion in the P(3HB-*co*-4HB). When the content of PLLA was as low as 40 wt%, the holes of the porous P(3HB-*co*-4HB) became bigger and bigger with the increasing content of PLLA. When the content of the PLLA increased to 40~60 wt%, there was a co-continuous phase appeared. However, the morphology of the PLLA in the P(3HB-*co*-4HB) was slightly different from that of P(3HB-*co*-4HB) in PLLA. When the content of the PLLA was 10 wt%, 20 wt%, and 30 wt%, the size of the porous P(3HB-*co*-4HB) was about 0.61, 1.73, and 3.14 μm, respectively. While when the content of the P(3HB-*co*-4HB) was 10 wt%, 20 wt%, and 30 wt%, the size of the porous PLLA was about 0.39, 1.08, and 2.65 μm, respectively. The size of the porous P(3HB-*co*-4HB) was slightly bigger than that of the porous PLLA. That meant the

P(3HB-*co*-4HB) was preferentially distributed in the PLLA. When the content of the P(3HB-*co*-4HB) were 40~60 wt%, the interconnected porous structure could be acquired through the selective enzymatic degradation and the morphology can be different due to the different content of P(3HB-*co*-4HB).

Accordingly, we can acquire the tunable porous materials with well-defined pore sizes, controlled pore size distributions, and interconnectivities through changing the composition and the content of the biodegradable component in the blends by selective biodegradation. The porous materials obtained are biodegradable, biocompatible and innocuous, which is expected to apply in tissue engineering area in future.

4. Conclusions

Fully biodegradable polymer blends of PLLA and P(3HB-*co*-4HB) were prepared by melt compounding. The enzymatic degradation and porous morphology of PLLA/P(3HB-*co*-4HB) blends were investigated in detail. The enzymatic degradation rate of P(3HB-*co*-4HB) was accelerated due to the addition of the PLLA which increased the specific surface area of the P(3HB-*co*-4HB), and the blends containing 30 wt%, 40 wt%, 50 wt% of PLLA had the most fast enzymatic degradation rate. For the samples with the content of 60 wt%, 80 wt% PLLA, the degradation rates were decreased due to the package effect of PLLA-rich phase on the P(3HB-*co*-4HB). For the enzymatic degradation of PLLA, the degradation rate was also improved due to the similar effect. The composition changes in the PLLA and P(3HB-*co*-4HB) characterized by ^{1}H NMR could be matched well with the corresponding weight loss of the PLLA/P(3HB-*co*-4HB) films after the enzymatic degradation ether by the *Pseudomonas mendocina* lipase or proteinase K catalyzed. The results of the ^{1}H NMR and GPC indicated that there were no intermediate products formed during the enzymatic degradation both of the PLLA and P(3HB-*co*-4HB), which confirmed the surface erosion mechanisms for the

degradation of the PLLA and P(3HB-*co*-4HB) catalyzed by the proteinase K and *Pseudomonas mendocina* lipase, respectively. The selective enzymatic degradation results revealed that when the P(3HB-*co*-4HB) component was degraded, the pore in the PLLA became more and more bigger with increasing the P(3HB-*co*-4HB)content, and it became interconnected pores when the content of the P(3HB-*co*-4HB) increased to 40~60 wt%. There was a similar phase inversion in the porous P(3HB-*co*-4HB). However, it should be noted that the size of the porous PLLA was slightly smaller than that of the porous P(3HB-*co*-4HB), indicating the P(3HB-*co*-4HB) was preferentially distributed in the PLLA. Consequently, the porous materials could be obtained through the selective biodegradation method and the morphology of the pore can be controlled through changing the composition and the content of the biodegradable component in the blends. The porous materials fabricated have tunable mechanical properties, well-defined pore sizes, and controlled pore size distributions and interconnectivities. Since both of the materials for blending are environmentally friendly, the porous materials obtained are biodegradable, biocompatible and innocuous whether the second component is removed completely or not, which are suitable for the application of tissue engineering. Such studies may be of great interest and importance for the development of scaffolds for regeneration of tissues from biodegradable polymers.

Acknowledgements

The authors are grateful for the support from the National Natural Science Foundation of China (51021003).

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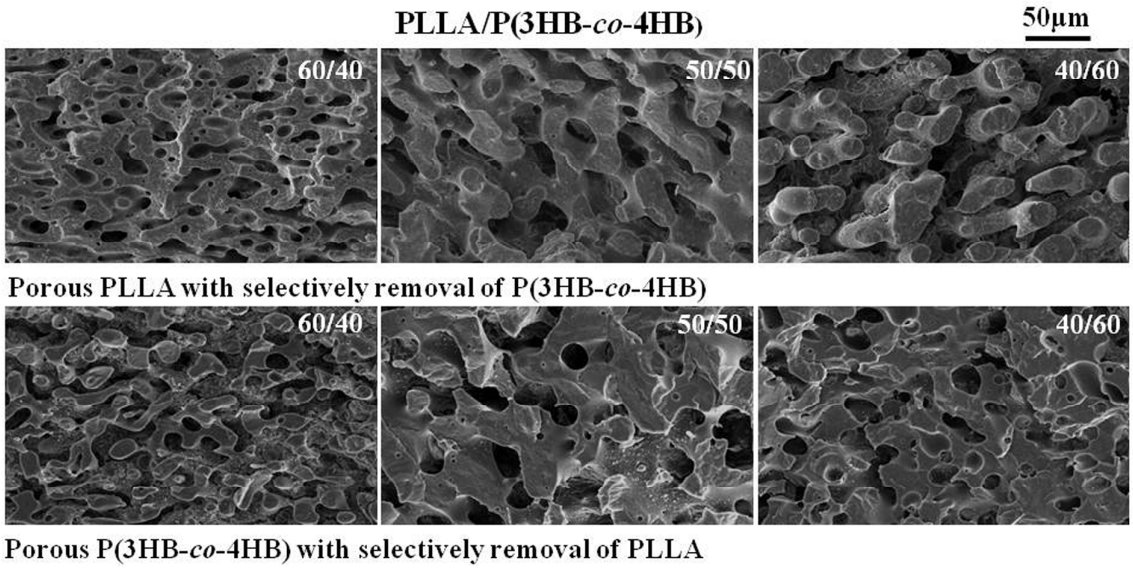
Table of Contents Graphic



Due to the specifity of the degradation enzymes, the porous PLLA and the porous P(3HB-*co*-4HB) could be acquired by selectively removal of the P(3HB-*co*-4HB) component and the PLLA component, respectively.