Thermal hydrolysis of poly(L–lactic acid) films and cytotoxicity of water–soluble degradation products

9 AUTHORS, INCLUDING:

xu Guoguang
Chinese Academy of Sciences
3 PUBLICATIONS   **4** CITATIONS

SEE PROFILE

Zhong Liu
Jinan University (Guangzhou, China)
35 PUBLICATIONS   **330** CITATIONS

SEE PROFILE

JH Zhao
Jinan University (Guangzhou, China)
31 PUBLICATIONS   **215** CITATIONS

SEE PROFILE

Thermal hydrolysis of poly(L-lactic acid) films and cytotoxicity of water-soluble degradation products

Guoguang Xu,[1] Xiao Liu,[2] Yanqun Lin,[1] Guoshan He,[3] Wanjuan Wang,[3] Wen Xiong,[1] Haiying Luo,[3] Zhong Liu,[4] Jianhao Zhao[1]

[1]Department of Materials Science and Engineering, College of Science and Engineering, Jinan University, Guangzhou 510632, China
[2]Guangdong Key Laboratory of Pharmaceutical Bioactive Substances, Guangdong Pharmaceutical University, Guangzhou 510006, China
[3]Guangzhou Product Quality Supervision and Testing Institute, Guangzhou 510725, China
[4]Guangzhoujinan Biomedicine Research and Development Center, Guangdong Provincial Key Laboratory of Bioengineering Medicine, National Engineering Research Center of Genetic Medicine, Jinan University, Guangzhou 510632, China
Correspondence to: Z. Liu (E-mail: tliuzh@jnu.edu.cn) and J. Zhao (E-mail: jhzhao@jnu.edu.cn)

ABSTRACT: In this study, the thermal hydrolysis of the poly(L-lactic acid) (PLLA) films was investigated for its potential use as a food-packaging ecomaterial. The surface morphology, mass loss, molecular weight, thermal properties, and medium pH were routinely investigated; meanwhile, in particular, the composition and cytotoxicity of the water-soluble degradation products were studied. The changes in the mass loss and molecular weight revealed a random chain-scission mechanism. Differential scanning calorimetry analysis implied that the hydrolysis preferentially took place in the amorphous region. The medium pH decreased with time because of the accumulation of acid water-soluble products in the medium. Liquid chromatography/mass spectrometry analysis proved that these products were composed of 1–13 lactic acid units, in which the content of L-lactic acid increased with time and reached 9.71 mmol/L after hydrolysis for 84 days. The *in vitro* cell culture indicated that the water-soluble degradation products from the PLLA films had no cytotoxicity to human umbilical vein endothelial cells. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42064.

INTRODUCTION

With the increasing use of polyolefin materials, tens of millions of plastics are discarded every year, and this leads to global environmental pollution and resource shortage.[1,2] These waste plastics primarily come from the packaging industry, in which food packaging occupies most of the products, including beverage bottle, cling film, packing bags, and so on.[3–5] Landfill and incineration are two conventional ways for these wastes; however, these treatments also cause harm to the environment by occupying lots of lands or emitting toxic gases.[6,7] To solve this problem, one solution is to develop biodegradable polymers that can be degraded by microorganisms to nontoxic carbon dioxide, water, and metabolites in the soil.[1,8]

Many biodegradable polyesters, such as polylactides, poly(butylene succinate), poly(butylene succinate-*co*-butylene adipate), and poly(ε-caprolactone), have been widely studied as ecomaterials in the plastics-packaging industry.[8–15] Under the conditions of radiation, heat, microorganisms, or humidity, these polyesters can be degraded to small fragments that may depart from the polymer matrix and diffuse into the surroundings.[16,17] Among various biodegradable polyesters, poly(lactic acid) (PLA) has attracted much interest for its renewable raw material, high melting point, good mechanical properties, and easy fabrication and has been considered as one of the most promising biodegradable materials in the food-packaging field.[18–21] However, when PLA is in long-term use in food packaging, especially aqueous food packaging, PLA will be inevitably degraded by the environment because of its degradability. In that case, the water-soluble degradation products from PLA, including lactic acid and other small oligomers, probably migrate to the food and cause contaminations.[22,23] Although lactic acid has been used in food for a long time and is considered safe as an intentional food ingredient or when naturally occurring in food,[24] recent research has found that the cytotoxicity is significant when the concentration of lactic acid is as high as 20 mmol/L.[25]

When PLA is used in long-term food packaging, it is anticipated that the lactic acid content will increase with time; meanwhile, other water-soluble small oligomers of lactic acid also coexist, although they will eventually decompose into lactic acid. Whether these small molecular oligomers and accumulative lactic acid monomers are harmful or not is unknown. Hence, it is a worthy cause to look into the cytotoxicity of both the accumulative lactic acid and the intermediate small oligomers from the degradation of PLA as a promising food-contact-packaging ecomaterial.

PLA has three isomers, that is, poly(D-lactic acid), poly(D,L-lactic acid), and poly(L-lactic acid) (PLLA), among which PLLA has been most widely used because of its better mechanical and processing properties.[26] To obtain the water-soluble degradation products of PLLA, hydrolysis is a simple way and includes thermal hydrolysis, acid hydrolysis, alkali hydrolysis, and enzymatic hydrolysis.[27–29] In comparison to other types of hydrolysis, thermal hydrolysis can better reflect the real degradation of PLLA in food packaging. In this study, thin PLLA films with a thickness of 150 μm approximate to the thickness of commercial beverage bottles were chosen for thermal hydrolysis. It has been reported that a higher temperature above the glass-transition temperature (T_g) will affect the viscoelastic behavior of the polymer matrix and, therefore, enhance the diffusion of inclusions.[30,31] PLLA possesses a T_g of 60–65°C depending on the isotacticity of the polymer chain; thus, the selection of a hydrolysis temperature of 70°C, which is slightly higher than T_g, can not only accelerate the hydrolysis of PLLA but also favors the diffusion of water-soluble degradation products. In addition to the routine investigations on surface morphology, mass remaining percentage, molecular weight, thermal properties, and medium pH, the water-soluble degradation products were analyzed by ultraperformance liquid chromatography (UPLC)/mass spectrometry (MS). The *in vitro* cytotoxicity of these products to human umbilical vein endothelial cells (HUVECs) was also evaluated.

EXPERIMENTAL

Materials
PLLA (medical grade), with a weight-average molecular weight (M_w) of 4.0×10^5 Da, a crystallinity (X_c) of 43.8% and a polydispersity of 1.90, was provided by Shandong Institute of Medical Instruments (China). L-Lactic acid was purchased from Sigma-Aldrich. All of the other reagents and solvents used were analytical grade.

Preparation of the PLLA Films
PLLA films were prepared by a casting method as in our previous work.[32] Briefly, a 0.03 g/mL PLLA solution in chloroform was cast in a glass dish (the diameter, $\Phi = 150$ mm). The dish was subsequently placed in a hood for 2 days; this allowed the solvent to evaporate slowly at room temperature. After the residue solvent was removed *in vacuo* (0.5 mmHg) for 48 h, the film was obtained with a thickness of about 150 μm, which was approximate to the wall thickness of commercial beverage bottles. Before use, the film sheet was cut into 1×1 cm^2 pieces.

Thermal Hydrolysis of the PLLA Films
PLLA films of approximately 60 mg were soaked in 5 mL of deionized water in each glass tube. The tube was sealed with a rubber plug and then transferred to an oven where the hydrolysis of PLLA films was statically performed at 70°C for 84 days. At desired intervals, the films were removed from the aqueous solution and dried *in vacuo* to a constant weight for various characterizations. For every time interval, three reduplicate tests were performed in parallel.

Morphology Observation
The surface morphology of the PLLA films after hydrolysis was observed by scanning electron microscopy (SEM; Philips XL-30ESEM, The Netherlands) with an accelerating voltage of 20 kV. Before SEM analysis, a gold layer was coated on the specimen surface by a sputter coater (BAL-TEC, SCD005, Finland).

Weight Remaining Percentage Analysis
The weight remaining percentage of the PLLA films after hydrolysis was measured gravimetrically. At desired intervals, the PLLA films were taken out from the medium and dried to constant weight *in vacuo* (0.5 mmHg) at 50°C in an oven. The weight remaining percentage was evaluated as follows:

$$W_{r,t}\% = W_t / W_0 \times 100\%$$

where $W_{r,t}\%$ is the percentage of weight remaining in the PLLA film at time t, W_t is the residual weight of PLLA film at time t, and W_0 is the original weight of the PLLA film before hydrolysis.

Molecular Weight Analysis
The molecular weight of the PLLA films during hydrolysis was measured by a gel permeation chromatography (GPC) instrument (Waters) equipped with a Waters 1515 pump, a Waters 2414 refractive-index detector, two Agilent PLgel 10-μm Mixed-B columns and a Breeze 2 UPLC system. Chromatographically, pure chloroform was used as the mobile phase at a flow rate of 1.0 mL/min. Polystyrene standards with various molecular weights were used to plot the calibration curve.

Thermal Property Analysis
The thermal properties of the PLLA films during hydrolysis were studied by differential scanning calorimetry (DSC; Netzsch DSC 204F1 Phoenix, Germany). Samples of about 6 mg were placed in an aluminum crucible and then sealed by a lid. The samples were first heated from 0 to 200°C at 10°C/min under a nitrogen gas flow of 50 mL/min to remove the thermal history, then cooled to 0°C at 10°C/min, and reheated to 200°C again at 10°C/min. The second heating curve was used for the analysis.

Medium pH Measurement
The aqueous medium containing the water-soluble degradation products of the PLLA films was collected at desired intervals, and then, the pH value was measured with a pH meter (Mettler Toledo, Shanghai, China).

Water-Soluble Degradation Product Analysis
The water-soluble degradation products analysis was performed with a UPLC/MS instrument (Waters UPLC XEVO TQMS). Briefly, the aqueous solution containing water-soluble degradation products was collected after filtration with a 0.22-μm filter. After 25 dilutions, 20 μL of diluent was injected into the UPLC system equipped with a Waters Spheisorb C$_{18}$ column (250×4.6 mm^2,
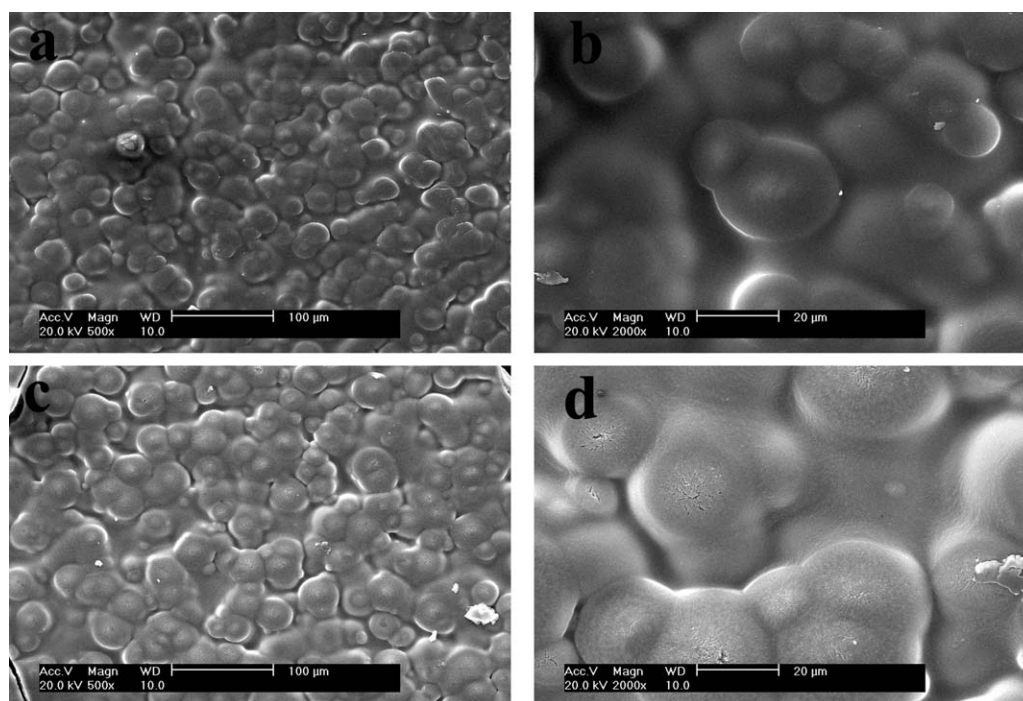
Figure 1. SEM images of PLLA film surfaces (a,b) before and (c,d) after hydrolysis for 35 days in deionized water at 70°C.

5 μm) and an ultraviolet detector using the maximum absorption wavelength of 206 nm. A mobile phase of 0.1% acetic acid/acetonitrile (70:30 v/v) at a flow rate of 0.2 mL/min was used. A calibration curve of L-lactic acid with known concentration was used to quantify the content of L-lactic acid monomer in the medium. The mass spectra were recorded with an electrospray ionization source in a positive mode. The operation parameters were as follows: capillary voltage = 2.5 kV, cone voltage = 20 V, source temperature = 150°C, desolvation temperature = 350°C, cone gas flow = 50 L/h, and desolvation gas flow = 650 L/h. Under these conditions, the positive molecular ion peaks of the water-soluble degradation products were expected to appear. The degradation fragments (fragment molecules plus Na^{+}) with a mass–charge ratio (m/z) range of 0–1000 were monitored by a selected ion-monitoring technique.[33]

Cytotoxicity Analysis
At various intervals, 600 μL of the degrading medium was lyophilized at 0.5 mmHg and redissolved in 600 μL of the endothelial cell medium; this was followed by filtration with a 0.22-μm filter membrane for sterilization. HUVECs at a density of 4000 cells per well were seeded in a 96-well plate containing 100 μL of endothelial cell medium per well. After 24 h of incubation in an incubator with 5% CO_2 at 37°C, the supernatant was removed, and 100 μL of degradation products in endothelial cell medium was added and cultured for 48 h. A cell counting kit 8 (10 μL) was then added to each well. After 4 h, the optical density at 450 nm was recorded by an automatic enzyme scanner (Bio-Rad 680). The morphology of the HUVECs was observed with an inverted light microscope (Olympus IX71, Japan). In this experiment, the well with the previous operation but without degradation products was used as the control.

Statistical Analysis
Data are given as the means plus or minus standard deviation ($n = 3$). To test the significance of observed differences between the study groups, the Student's t test was applied. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Surface Morphology
It is well known that aliphatic polyesters containing ester bonds in the polymer chain can be hydrolyzed by water molecule attack. In particular for a film sample, this interaction will first happen on the surface because of the direct contact with the medium. Thus, the surface morphology of the PLLA films was supposed to change when the hydrolysis was initiated. The SEM images of the PLLA film surface before and after hydrolysis are shown in Figure 1. For the original PLLA films, many round hunches were observed; these were due to the formation of a spherocrystal structure on the side facing the air during the slow evaporation of the solvent.[34] After hydrolysis for 35 days, the border of these spherical crystallites became obscure and accompanied some small cracks, but the films became frangible, as shown in the digital photo of Figure 2(c). When the time was prolonged to 42 and 84 days, the films turned pale and were very easy to break into debris [Fig. 2(d)], so it was difficult to obtain their SEM images. These results indicate that the hydrolysis of the PLLA films took place.

Weight Percentage Remaining and Molecular Weight
The degradation of the polymers usually accompanies changes in the mass and molecular weight, except for the surface morphology. The changes in the weight percentage remaining and molecular weight of the PLLA films during hydrolysis were
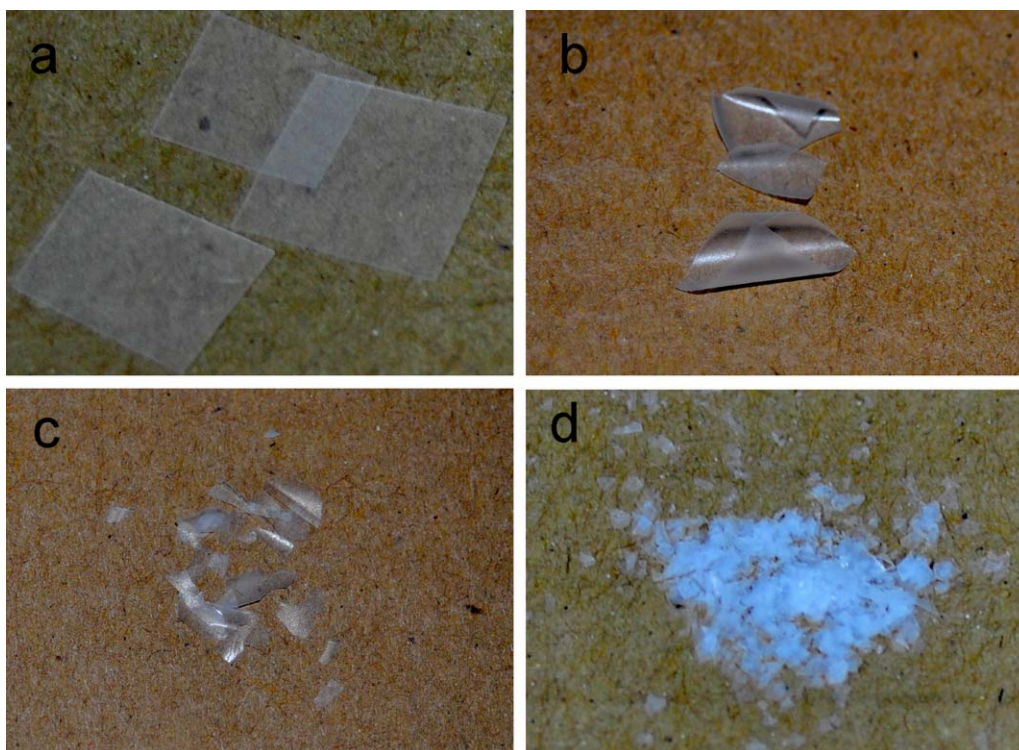
Figure 2. Digital photographs of the PLLA films after hydrolysis for different times in deionized water at 70°C: (a) 0, (b) 7, (c) 35, and (d) 84 days. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

further investigated by gravimetrical measurement and GPC analysis, respectively, as shown in Figure 3. During the early period, the M_w value dropped sharply from the original $4.0 \pm 0.1 \times 10^5$ to $2.1 \pm 0.8 \times 10^4$ Da at 35 days; this indicated a 95% reduction in M_w. After that, this descent started to decelerate, and a final M_w of $8.1 \pm 1.0 \times 10^3$ Da at 84 days was obtained. In contrast, the residual weight of the PLLA films decreased slowly with time. In the first week, the approximate 8% weight loss was probably from the quick degradation and diffusion out of small parts and amorphous regions on the surface of PLLA films. However, during the periods of 7 and 35

Figure 3. Weight remaining percentages and M_w values of the PLLA films during hydrolysis in deionized water at 70°C ($n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

days, there was no significant weight loss, even though M_w kept decreasing; this may have been because most of the degradation products were still too large to dissolve in the medium. Meanwhile, the static incubation in this study might have also slowed down the migration of small fragments into the medium. It has been reported that the weight loss of polylactides is generally not observed until the molecular weight decreases to 15,000 or less.[35] After 35 days, the weight loss became faster, but only approximately 20% was lost at 84 days; this suggested that most of the fragments remained in the substrate. This speculation was confirmed by the GPC traces of the PLLA films during hydrolysis, as recorded in Figure 4. Before 28 days, a single peak was observed at a low elution time; this indicated that the molecular weight was large and relatively homogeneous. However, from 35 days on, obvious double peaks appeared at a higher elution time; this suggested that the molecular weight decreased and was heterogeneous. These typical double peaks in the GPC patterns of polyester degradation generally contributed to the formation of small fragments remaining in the substrate.[11] These characteristic changes in the mass and molecular weight indicated a random scission mechanism for PLLA hydrolysis.[36] In the early period, the PLLA chains were randomly broken to fragments, and this led to the rapid reduction of molecular weight, but most of the fragments were still water-insoluble and remained in the substrate. This resulted in the low mass loss. With increasing degradation time, these fragments were further degraded to smaller products until water-soluble oligomers and monomers were formed; these could more easily migrate to the medium from the substrate and so caused a faster mass loss in the late period.
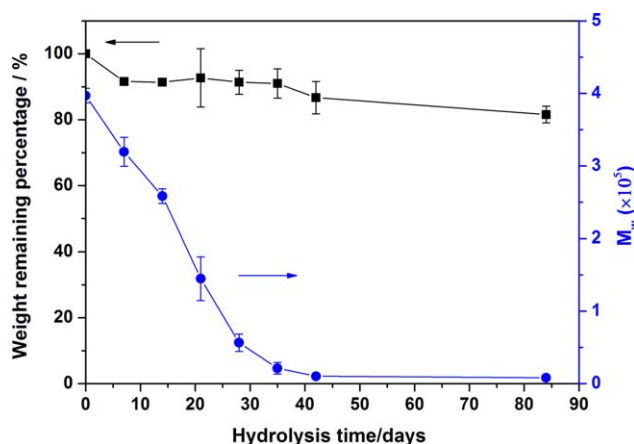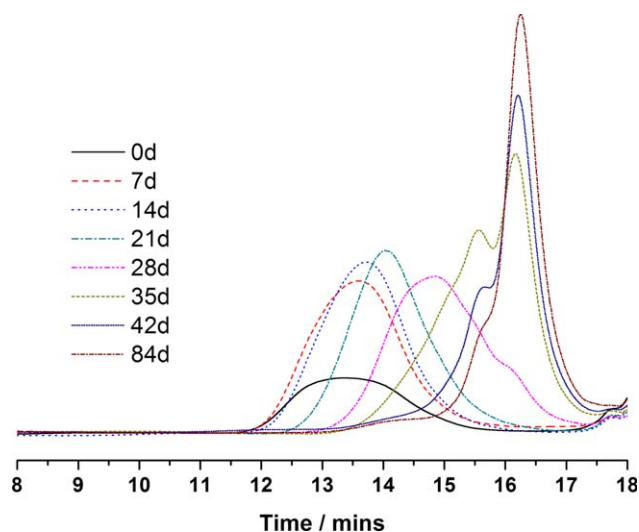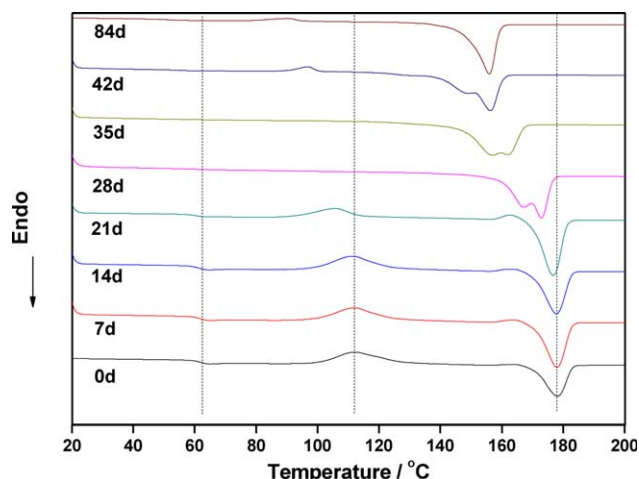
Figure 4. GPC traces of the PLLA films during hydrolysis in deionized water at 70°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Thermal Properties

The thermal properties of the PLLA films after hydrolysis were studied by DSC analysis, as presented in Figure 5 and summarized in Table I. Before hydrolysis, three characteristic peaks of PLLA were observed at 62, 112, and 178°C and were assigned as the T_g, cold crystallization temperature (T_c), and melting temperature (T_m), respectively.[37,38] With increasing degradation time, all of these peaks shifted to the low-temperature side, maybe because of the degradation of PLLA. In particular, the melting peak started to split into two peaks (166 and 173°C at 28 days); this could have been due to the formation of two types of crystallites with different thicknesses.[38,39] The thinner one melted at a lower temperature, whereas the thicker one melted at a higher temperature. However, as time went on, the relative peak intensity of the thinner crystallite decreased faster than the thicker one and even disappeared at 84 days; this may have been to its faster degradation. It was also notable that the

Table I. Thermal Properties of the PLLA Films During Hydrolysis by DSC Analysis

Degradation time (days)	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
0	62	112	178	41.0	43.8
7	62	112	178	57.1	61.0
14	61	111	177	55.2	59.0
21	60	105	176	67.4	72.1
28	-	-	173, 166	71.5	76.4
35	-	-	162, 156	77.8	83.2
42	-	97	156, 148	66.9	71.5
84	52	89	156	63.1	67.5

A dash indicates that the peak was not observed in the DSC curve. X_c of PLLA was calculated as follows:
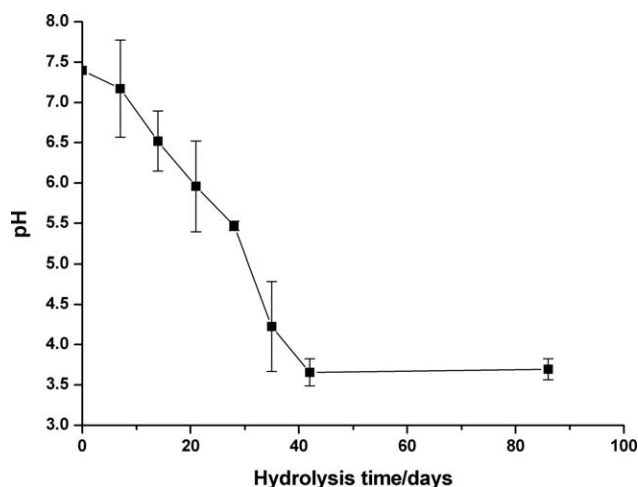
$$X_c = (\Delta H_m / \Delta H_m^\circ) \times 100\%$$

where X_c is the calculated crystallinity of the PLLA film, ΔH_m is the real melting enthalpy, and ΔH_m° (93.6 J/g) is the theoretical melting enthalpy for 100% crystalline PLLA.

calculated X_c of the PLLA films during hydrolysis initially increased and then dropped with a maximum value of 83% at 35 days. It was possible that the hydrolysis of the PLLA films preferentially happened in the amorphous region and induced the increase in X_c before 35 days, and the subsequent hydrolysis of the crystallites led to the decrease in X_c.[22]

Medium pH

On the basis of the previous analyses, we concluded that the hydrolysis of the PLLA films did take place and that the water-soluble degradation products should have entered the medium. The effect of these products on the medium was evaluated by the monitoring of the pH value, as shown in Figure 6. The pH value declined from 7.4 to 3.6 in the early 42 days and was then kept constant until 84 days. The reduction in the pH value was attributed to the formation of acid products that were dissociated from the films and dissolved in the medium. With increasing degradation time, more and more acid water-soluble

Figure 5. Second-heating DSC patterns of the PLLA films during hydrolysis in deionized water at 70°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 6. pH value of the degrading medium during the hydrolysis of the PLLA films in deionized water at 70°C ($n = 3$).
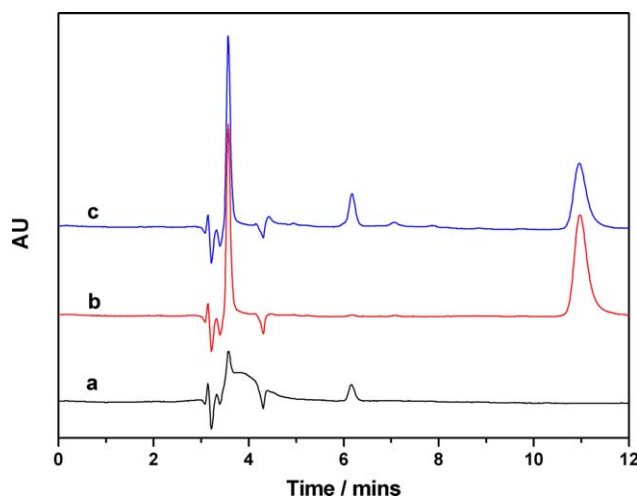
Figure 7. UPLC patterns of the degrading medium containing water-soluble degradation products from the hydrolysis of the PLLA films: (a) L-lactic acid at 2.00 mmol/L in deionized water as the control, (b) degrading medium at 7 days, and (c) degrading medium at 84 days. AU, Absorbance Unit. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Calculated Concentrations of the L-Lactic Acid Monomer and Relative Contents of the Lactic Acid Oligomers at Different Degradation Times from the UPLC Patterns

Degradation time (days)	Concentration of the L-lactic acid monomer (mmol/L)[a]	Relative content of the lactic acid oligomers[b]
7	0.08	1.0
14	0.40	1.1
21	0.72	124.2
28	0.96	12.2
35	1.33	5.1
42	6.28	1.4
84	9.71	0.2

[a] The concentration of L-lactic acid in the degrading medium was quantified according to the calibration curve ($y = 0.8479x + 0.1876$, $R^2 = 0.9999$) of L-lactic acid at known concentrations on the basis of the peak area at 6.2 min.
[b] The relative content of the lactic acid oligomers with time was calculated on the basis of the peak area at 11.0 min with the sample at 7 days as the control.

degradation products accumulated; this caused the continuous decrease of the pH value. Under acid conditions, the thermal hydrolysis rate of the PLLA films accelerated; this led to the distinct decrease in molecular weight (Fig. 3). However, the slow weight loss because of the slow migration of degradation fragments remaining in the substrate, which were proven by the appearance of double peaks in the GPC patterns, greatly slowed the decrease in pH after 42 days, even though the thermal hydrolysis of the PLLA films at 84 days was very severe, as shown by their appearance in the photos of the PLLA films in Figure 2.

Water-Soluble Degradation Products

To clarify these water-soluble degradation products from PLLA films, an UPLC/MS method was used for separation and identification. The UPLC patterns of the degrading medium at desired intervals are shown in Figure 7. At 84 days, in addition to the solvent peak at 3.6 min, two main peaks were observed at 6.2 and 11.0 min, respectively [Fig. 7(c)]. The peak at 6.2 min was assigned to the L-lactic acid monomer with L-lactic acid as the control [Fig. 7(a)], whereas the peak at 11.0 min was from the lactic acid oligomers because they showed a lower hydrophilicity than L-lactic acid that later eluted according to the relative polarities of the solid phase and mobile phase in
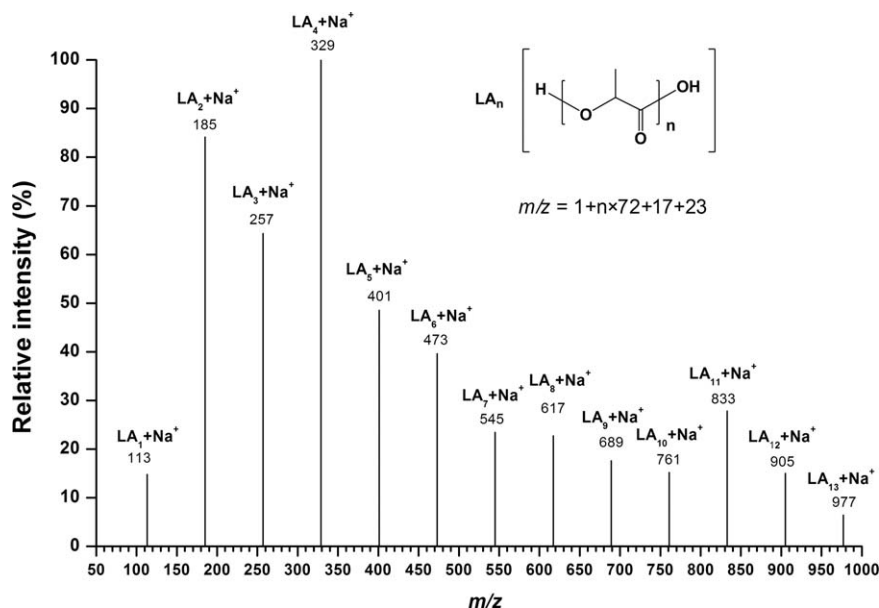
Figure 8. Mass spectrum of the water-soluble degradation products from the hydrolysis of the PLLA films for 84 days by a selected ion-monitoring technique with a positive electrospray ionization source (plus Na^+). Fragments with an m/z range of 0–1000 were monitored.
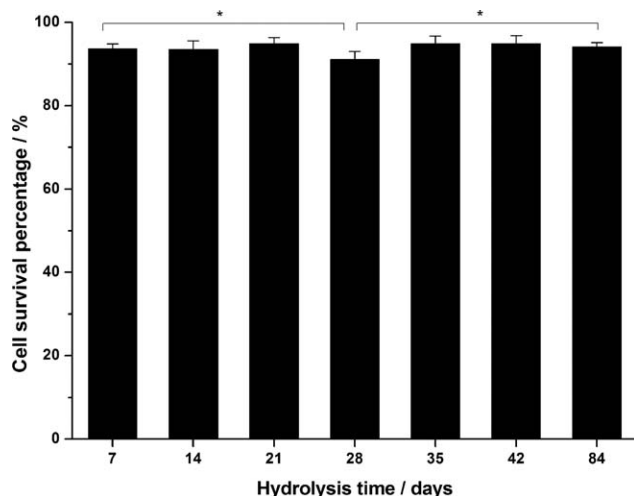
Figure 9. Viability of the HUVECs in the presence of water-soluble degradation products from the hydrolysis of the PLLA films in deionized water at 70°C for different times. The well without degradation products was used as the control. The optical density of various wells at 450 nm was measured, and the cell survival percentage was calculated as OD_{exp}/OD_{contr} ($n = 3$), $^{*}p < 0.05$.

this UPLC system. With L-lactic acid with known concentrations for calibration, the content of L-lactic acid in the medium could be quantified on the basis of its peak area at 6.2 min, which was proportional to the concentration of L-lactic acid. We found that with increasing degradation time, the calculated concentration of L-lactic acid increased and reached 9.71 mmol/L at 84 days; moreover, the relative content of oligomers calculated from the relative peak area at 11.0 min initially increased and then decreased (Table II). These results indicate in the early period that the hydrolysis of PLLA films preferentially produced lactic acid oligomers, which were further degraded to L-lactic acid as time went on. The continuously accumulative L-lactic acid in the medium led to the sharp decline in the pH, as shown in Figure 6. Although it was believable that the lactic acid oligomers were not from the single component, unfortunately, they could not be availably separated under these separation conditions, maybe because of their close polarities. For that reason, a selected ion-monitoring mode of MS was used to

quantify these water-soluble degradation products. Here, a positive electrospray ionization source with a low cone voltage of 20 V was used, and the molecular ion peaks of these small fragments were supposed to be obtained, as shown in Figure 8. The lactic acid oligomers were observed as one series of peaks with a successive m/z increase of 72 Da; this was the molar mass of the LA repeating unit. The peaks appearing at $m/z = (1 + n \times 72 + 17 + 23)$ corresponded to the lactic acid oligomers terminated with hydroxyl and carboxyl end groups plus one sodium ion each. The results show that these water-soluble degradation products were composed of 1–13 lactic acid units, that is, LA_1–LA_{13}. Unfortunately, because of the instability and lack of corresponding standard substances for quantification, these water-soluble lactic acid oligomers were unable to be quantified.

Cytotoxicity

To evaluate the cytotoxicity of these water-soluble degradation products for the potential application of PLLA as a food-packaging material, the survival percentage of HUVECs in the presence of water-soluble degradation products at various intervals was studied, as shown in Figure 9. Here, the cell survival percentage was defined as OD_{exp}/OD_{contr}, where OD_{exp} is the optical density detected from the experimental group and OD_{contr} is optical density detected from the control group. According to the international standard ISO 10993-5:2009(E), there were six cytotoxicity grades, which were defined as 0, 1, 2, 3, 4, and 5 and corresponded to cell survival percentages of 100, 75–99, 50–74, 25–49, 1–24, and 0%, respectively.[40] Grades 0 and 1 were considered to have no cytotoxicity. From Figure 9, the cell survival percentage for all samples was located at 93–95%; this suggested that the water-soluble degradation products from the PLLA films had no cytotoxicity to HUVECs. The morphology of the HUVECs for 48 h in culturing in the presence of water-soluble degradation products was also observed by an inverted light microscope, as shown in Figure 10. Similar to the control group, the HUVECs in the experimental groups grew well and exhibited a characteristic spindle shape; this was further evidence for the safety of water-soluble degradation products from the hydrolysis of the PLLA films to HUVECs. Actually, there was only one side of the PLLA films that would contact the aqueous food in use, so the concentration of L-lactic acid and its oligomers would
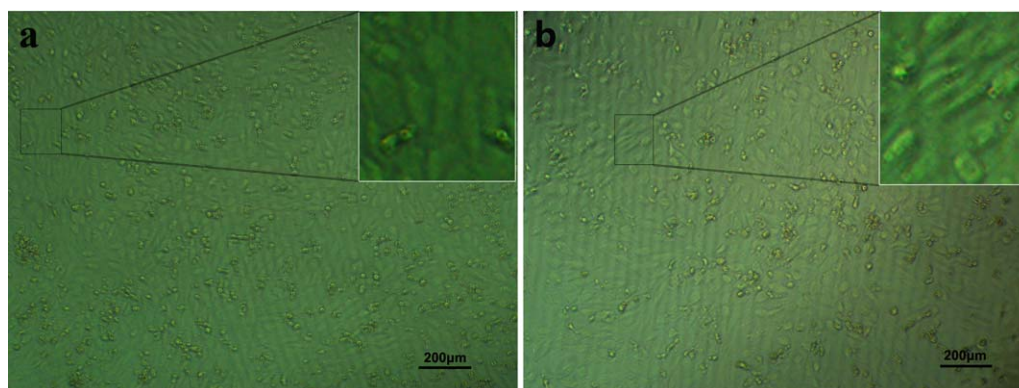
Figure 10. Optical micrographs of the HUVECs after they were cultured for 48 h (100×): (a) control group (HUVECs were treated without water-soluble degradation products) and (b) experimental group (HUVECs were treated with water-soluble degradation products from the hydrolysis of the PLLA films for 84 days). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

be reduced by half. Thus, it is more believable that PLLA is safe for potential applications as a food-packaging ecomaterial.

CONCLUSIONS

In this study, we aimed to investigate the thermal hydrolysis of PLLA films and illustrate the composition and cytotoxicity of water-soluble degradation products for the potential application of PLLA in food packaging. The thermal hydrolysis of PLLA films preferentially occurred in the amorphous region by a random chain-scission mechanism; this led to the formation of rough surface and X_c changes. The water-soluble degradation products from PLLA films migrated to the medium and caused a sharp decline in the pH value. These products were composed of 1–13 lactic acid units and had no cytotoxicity to HUVECs. It seems that PLLA is safe for potential applications as a food-packaging ecomaterial.

REFERENCES

1. Leja, K.; Lewandowicz, G. *Polym. J. Environ. Stud.* **2010**, *19*, 255.

2. Shimao, M. *Curr. Opin. Biotechnol.* **2001**, *12*, 242.

3. Silvestre, C.; Duraccio, D.; Cimmino, S. *Prog. Polym. Sci.* **2011**, *36*, 1766.

4. Barlow, C. Y.; Morgan, D. C. *Resour. Conserv. Recycl.* **2013**, *78*, 74.

5. Jayasekara, R.; Harding, I.; Bowater, I. *J. Polym. Environ.* **2005**, *13*, 231.

6. Khoo, H. H.; Tan, R. B. H. *Int. J. Life Cycle Assess.* **2010**, *15*, 338.

7. Brems, A.; Baeyens, J.; Dewil, R. *Therm. Sci.* **2012**, *16*, 669.

8. Vroman, I.; Tighzert, L. *Materials* **2009**, *2*, 307.

9. Widiastuti, I.; Sbarski, I.; Masood, S. H. *J. Appl. Polym. Sci.* **2014**, *131*, 40600.

10. Zhao, J. H.; Wang, X. Q.; Zeng, J.; Yang, G.; Shi, F. H.; Yan, Q. *J. Appl. Polym. Sci.* **2005**, *97*, 2273.

11. Zhao, J. H.; Wang, X. Q.; Zeng, J.; Yang, G.; Shi, F. H.; Yan, Q. *Polym. Degrad. Stab.* **2005**, *90*, 173.

12. Castilla, C.; Más-Estellés, J.; Meseguer, D. J. M.; Escobar, I. J. L.; Marí, B.; Vidaurre, A. *Polym. Degrad. Stab.* **2012**, *97*, 1241.

13. Khan, R. A.; Salmieri, S.; Dussault, D.; Sharmin, N.; Lacroix, M. *J. Appl. Polym. Sci.* **2012**, *123*, 1690.

14. Vieira, A. C.; Guedes, R. M.; Tita, V. *J. Polym. Eng.* **2013**, *33*, 293.

15. Auras, R.; Harte, B.; Selke, S. *Macromol. Biosci.* **2004**, *4*, 835.

16. Hakkarainen, M. In Advances in Polymer Science; Albertsson, A. C., Ed.; Springer: Berlin, **2002**; Vol. *157*, p 113.

17. Gan, Z. H.; Yu, D. H.; Zhong, Z. Y.; Liang, Q. Z.; Jing, X. H. *Polymer* **1999**, *40*, 2859.

18. Gaurav, K.; Rafael, A.; Sher, P. S.; Ramani, N. *Polym. Test.* **2007**, *26*, 1049.

19. Seksan, P.; Pomthong, M.; Ruethai, T.; Pechda, W.; Tassaneewan, C.; Manit, N.; Ed, S. *J. Clean Prod.* **2014**, *65*, 539.

20. Fausto, G.; Vincenzo, P. *Environ. Prog. Sustain.* **2011**, *30*, 459.

21. Vibeke, K. H.; Claus, J. W.; Bente, D.; Grete, B. *Eur. Food Res. Technol.* **2002**, *214*, 423.

22. Auras, R.; Lim, L. T.; Selke, S. E. M.; Tsuji, H. Poly(lactic acid): Synthesis, Structure, Properties, Processing, and Applications; Wiley: Hoboken, NJ, **2010**.

23. Mutsuga, M.; Kawamura, Y.; Tanamoto, K. *Food Addit. Contam. A* **2008**, *25*, 1283.

24. Conn, R. E.; Kolstad, J. J.; Borzelleca, J. F.; Dixler, D. S.; Filer, L. J. J.; LaDu, B. N. J.; Pariza, M. W. *Food Chem. Toxicol.* **1995**, *33*, 273.

25. He, Y.; Wang, W. R.; Ding, J. D. *Chin. Sci. Bull.* **2013**, *58*, 2404.

26. Madhavan, N. K.; Nair, N. R.; John, R. P. *Bioresour. Technol.* **2010**, *201*, 8493.

27. Piemonte, V.; Gironi, F. *J. Polym. Environ.* **2013**, *21*, 275.

28. Jae, H.; Jung.; Moonhor, R.; Heesoo, K. *Catal. Today* **2006**, *115*, 283.

29. Lee, S. H.; Song, W. S. *Appl. Biochem. Biotechnol.* **2011**, *164*, 89.

30. Zolnik, B. S.; Leary, P. E.; Burgess, D. J. *J. Controlled Release* **2006**, *112*, 293.

31. Aso, Y.; Yoshioka, S.; Li, W. P. A.; Terao, T. *J. Controlled Release* **1994**, *31*, 33.

32. Shah, A. A.; Eguchi, T.; Mayumi, D.; Kato, S.; Shintani, N.; Kamini, N. R.; Nakajima, K. T. *Polym. Degrad. Stab.* **2013**, *98*, 2722.

33. Zhao, J. H.; Wang, J.; Tu, M.; Luo, B. H.; Zhou, C. R. *Biomed. Mater.* **2006**, *1*, 247.

34. Zhao, J. H.; Han, W. Q.; Tang, M. J.; Tu, M.; Zeng, R.; Liang, Z. H.; Zhou, C. R. *Mater. Sci. Eng. C* **2013**, *33*, 1546.

35. Pitt, G. G.; Gratzl, M. M.; Kimmel, G. L.; Surles, J.; Schindler, A. *Biomaterials* **1981**, *2*, 215.

36. Helder, J.; Dijkstra, P. J.; Feijen, J. *J. Biomed. Mater. Res. A* **1990**, *24*, 1005.

37. Yeh, J. T.; Tsou, C. H.; Lu, W.; Li, Y. M. *J. Polym. Sci. Part B: Polym. Phys.* **2010**, *48*, 913.

38. Cheng, S. J.; Yang, L. J.; Gong, F. R. *Polym. Bull.* **2010**, *65*, 643.

39. Kantoglu, O.; Guven, O. *Nucl. Instrum. Methods Phys. Res. B* **2002**, *197*, 259.

40. Chen, Y. M.; Xi, T. F.; Zheng, Y. D.; Guo, T. T.; Hou, J. Q.; Wan, Y. Z.; Gao, C. *J. Bioact. Compat. Polym.* **2009**, *24*, 137.