JOURNAL OF Applied Polymer SCIENCE

Regulating the Physical and Biological Performances of Poly(*p*-dioxanone) by Copolymerization with L-Phenylalanine

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ABSTRACT: The high crystallinity, low solubility in normal solvents, and low hydrophilicity of poly(*p*-dioxanone) (PPDO) are unsuitable for the expansion of its biomedical applications. In order to circumvent these problems and induce biological properties, a series of poly(ester amide)s based on *p*-(dioxanone) and L-phenylalanine were synthesized by copolymerization of *p*-dioxanone with L-phenylalanine *N*-carboxyanhydride monomers. The structures of the copolymers were confirmed by ^{1}H NMR. The crystallinity of the copolymers was investigated by differential scanning calorimetry (DSC) and polarized optical microscopy (POM). Increasing contents of phenylalanine resulted in decreased crystallinity owing to the rigid phenyl groups of phenylalanine, which disrupted the regularities of the chains, thus confining their movement. The synthesized copolymers were more soluble in chloroform than PPDO. Moreover, the copolymers were more hydrophilic and hydrolyzed more slowly than PPDO, as indicated by water angle contact measurements and *in vitro* hydrolysis studies. Especially, the copolymers showed inhibition on cell proliferation of L929 mouse fibroblasts by MTT assay, suggesting that the polymers might be useful in the areas where cell proliferation need to be inhabited such as adhesion prevention. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 2311–2319, 2013

KEYWORDS: biomaterials; copolymer; crystallization; *p*-(dioxanone); L-phenylalanine

Received 14 November 2013; accepted 23 April 2013; Published online 20 May 2013
DOI: 10.1002/app.39455

INTRODUCTION

Poly (*p*-dioxanone) (PPDO), as an aliphatic polyester, has been used in biomedical areas such as drug delivery,[1–4] vascular graft materials,[5] bone pins,[6] and cartilaginous tissue engineering[7] because of its outstanding biodegradability, biocompatibility, and flexibility. However, the intrinsic hydrophobicity along with its lack of specific biological properties have limited its further application in biomedical areas, like other aliphatic polyesters such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and poly (ε-caprolactone) (PCL). Moreover, the high crystallinity of PPDO results in poor solubility in normal organic solvents.

The mostly used method to improve the performances of PPDO, such as hydrophilicity, solubility and *in vitro* hydrolysis behavior, is the introduction of foreign component by copolymerization. This method is mainly based on two principles. One is the introduction of hydrophilic block components, such as PEG or poly (vinyl alcohol) to improve its hydrophilicity.[8–11] The other is the disturbance of crystallinity property of PPDO by introduction of randomly copolymerized components, which leads to a ameliorative solubility. It has been demonstrated that the crystallinity of Poly(epsilon-caprolactone-*co*-*p*-dioxanone) random copolymers and poly(D,L-lactide-*co*-*p*-dioxanone) copolymers could be controlled by adjusting the composition or copolymerization conditions.[12,13] However, those components copolymerized with PDO do not have biological properties and cannot change the cell-materials interreactions. Therefore, a method which could both change the physical properties and biological properties should be developed for the expension of PPDO's biomedical applications.

It is known that α-amino acids and their polymers exhibit better hydrophilicity and biomedical properties especially the cell adhesion and enzymatic degradation. Furthermore, the amino acid sequences could provide reactive functional groups with which the polymers could be modified with the arginine–glycine–aspartic acid (RGD) sequence or other genes and protein recognition groups, thereby promoting the cellular and biological response of the polymers.[14–29] In those amino acid series, L-phenylalanine is one of the most important ones. It is a

naturally occurring essential α-amino acid that may be assimilated by living organisms after degradation of polymers and can be effectively digested by α-chymotrypsin for its benzyl group. In addition, there are few researchers reported the specifically inhibitions of L-phenylalanine on the proliferation of rat myocardial fibroblast and smooth muscle cell.[30,31] It is an important and particular biological property of L-phenylalanine.

Therefore, we present here the design and synthesize a new kind of copolymer composed of PDO and amino acids which should possess both the biological properties and improved physical properties. In this study, L-phenylalanine was used as a template amino acid to design and synthesize the target copolymer because its rigid benzyl groups may change the flexibility of the copolymer chains and decrease the crystallinity along with its specifically inhibitions on cell proliferation. It is expected that performances of copolymer, such as the biological properties, the crystallinity, hydrophilicity, and *in vitro* hydrolysis property could be improved compared to PPDO. In particular, the copolymer could inhibit cell proliferation for the inhibition effect of L-phenylalanine. Therefore, the copolymer would have potential application in the areas where cell proliferation need to be inhabited such as adhesion prevention.

In this study, copolymers of poly(*p*-dioxanone-*co*-L-phenylalanine) (PDPA) with different L-phenylalanine contents were synthesized, and their crystallinity, hydrophilicity, and *in vitro* hydrolysis were also studied. In addition, the mouse L929 fibroblasts proliferations on their electrospun membranes were investigated too.

EXPERIMENTAL

Materials

The *p*-dioxanone (PDO) was synthesized by our group and used without further purification.[32] Amino acids (99%) were purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China) and dried under vacuum at room temperature for 24 h before use. Stannous octoate (95%) was purchased from Sigma-Aldrich and used without further purification. Chloroform (99%), diethyl ether (99%), THF (99%), and hexane (99%) were purchased from Chengdu Kelong Chemical Factory (Chengdu, China). THF was dried and distilled in the presence of sodium, hexane was dried and distilled in the presence of calcium hydroxide immediately before use, and other solvents were used as received.

Measurements

The ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AV300 spectrometer (Germany). Deuterated chloroform and deuterated dimethyl sulfoxide were used as the solvents with tetramethylsilane (TMS) as the chemical shift standard.

FTIR spectra were measured using a Thermo Fisher Scientific Nicolet 6700 spectrometer and samples were prepared using tablets of KBr.

The weight average molecular weight (M_w) of the macromolecules was determined by gel permeation chromatography (GPC) using a system consisting of a Waters 1515 isocratic HPLC pump, Styragel HT 4 or HT 5 column, and Waters 2414 refractive index detector. Chloroform was the eluent at a flow rate of 0.5 mL/min. Narrow molecular weight distribution polystyrene was used as the molecular weight basis.

Differential scanning calorimetry (DSC) was performed with a TA Instruments DSC (Model Q20). The samples were loaded in aluminum pans, heated to 130°C for 3 min to erase all thermal history, and then cooled to −40°C at a cooling rate of 10°C/min. After cooling the heating scan were followed from −40°C to 130°C at a heating rate of 10°C/min.

The crystal morphologies of PPDO and PDPA crystallized from the melted state were observed by POM using an XPN-203 equipped with a hot stage (Shanghai Changfang Optical Instrument Co.) and a TK-C921EC camera system (JVC, Japan). Thin films of PPDO and PDPA were prepared between cover slips by melting at 130°C for 5 min. The samples were then observed by cooling to the isothermal crystallization temperature of 55°C.

The hydrophilicity of PPDO and PDPA were evaluated based on the water static contact angles of their cast films (Krüss DSA 100 goniometer). The surface topography of PPDO and PDPA cast films were observed with an atomic force microscope (AFM) operating in tapping mode using an instrument with a SPI4000 Probe Station controller (SIINT Instruments, Japan) at room temperature. Height and phase contrast images were collected. Olympus tapping mode cantilevers with the spring constants ranging from 51.2 to 87.8 N/m (as specified by the manufacturer) were used with a scan rate in the range of 1.0–2.0 Hz.

Scanning electron microscopes (SEM) images were taken by model JSM-6300 microscope (Japan).

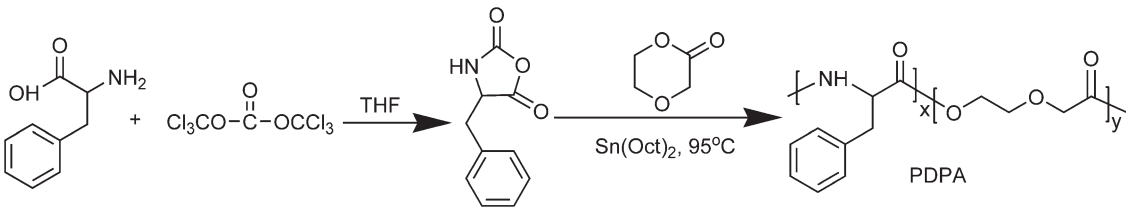
Synthesis of PPDO

To a 50-mL round-bottom flask containing PDO (10 g) and octadecanol (0.0475 g) was added stannous octoate (2 mg). The flask was sealed using a long-neck pipe connected to an oil pump to remove the moisture, oxygen, and solvent until the pressure of flask was reduced to 10^{-3} Pa below. The polymerization was carried out under vacuum at 110°C with magnetic stirring for 20 h. The crude product was dissolved in hexafluoroisopropanol and precipitated with diethyl ether twice. The purified product was then filtered and evaporated under vacuum at room temperature for 24 h. ^{1}H NMR (300 MHz, CD_3Cl, δ, ppm): 4.34 (*t*, CH_2OCO), 4.17 (*s*, $COCH_2O$), 3.79 (*t*, OCH_2), 1.28 (*m*, $(CH_2)_{16}$), 0.87 (*t*, CH_3). $M_n = 17878$, $M_w = 29895$, $M_w/M_n = 1.67$ (by GPC).

Synthesis of L-Phenylalanine N-Carboxyanhydride[33]

The L-Phenylalanine *N*-Carboxyanhydride (L-Phe-NCA) was synthesized from L-phenylalanine (5 g) and triphosgene (3.7 g) in dry THF (50 mL) at 50°C for 4 h under N_2 atmosphere. After completion of the reaction, the mixture was concentrated and dry hexane was added to precipitate the crude product. The crude product was purified by recrystallization from diethyl ether/hexane twice to yield L-Phenylalanine *N*-Carboxyanhydride monomer of high purity.

^{1}H NMR (300 MHz, DMDO-d_6, δ, ppm): 9.09 (*s*, 1*H*, NH), 7.15–7.33 (*m*, 5*H*, C_6H_5), 4.77 (*t*, 1*H*, CH), 3.01 (*d*, 2*H*, CH_2).



Scheme 1. Synthetic route of poly(*p*-dioxanone-co-L-phenylalanine).

^{13}C NMR (75 MHz, DMDO-d_6, δ, ppm): 170.84, 151.65, 134.79, 129.69, 128.43, 127.16, 58.24, 36.27.

FTIR (KBr, cm^{-1}): 3351 (N—H), 3031 (C—H from phenyl), 2905 (C—H), 1849 (C=O), 1779 (C=O).

Synthesis of PDPA

To a 100-mL round-bottom flask containing PDO (5 g) were added calculated quantities of L-Phenylalanine *N*-Carboxyanhydride and stannous octoate. The flask was sealed using a long-neck pipe connected to an oil pump to remove the moisture, oxygen, and solvent until the pressure was reduced to 10^{-3} Pa below. The polymerization was carried out under vacuum at 95°C with magnetic stirring for a calculated period of time. The crude product was dissolved in chloroform and then was filtrated to remove the possible existent of homopolymer. The filtrate was precipitated by diethyl ether, and then the precipitation was dissolved in chloroform again and precipitated with ethanol. The purified product was filtered and dried under vacuum at room temperature for 24 h. ^{1}H NMR (300 MHz, DMDO-d_6, δ, ppm): 8.00 (*d*, NH), 7.16–7.27 (*m*, C_6H_5), 4.58 (*m*, CH of L-Phenylalanine structure units), 4.20 (*t*, $COCH_2OCH_2\underline{CH_2}O$), 4.16 (*s*, $CO\underline{CH_2}OCH_2CH_2O$), 3.69 (*t*, $COCH_2O\underline{CH_2}CH_2O$), 3.05 (*m*, CH_2 of L-Phenylalanine structure units).

FTIR (KBr, cm^{-1}): 3413 (N—H), 2980–2881 (C—H), 1749 (C=O), 1678 (amide I), 1525 (amide II), 1137 (O—C—O).

Water Contact Angle Measurement and *In Vitro* Hydrolysis

The thin films of the copolymers for the water contact angle and *in vitro* hydrolysis measurements were prepared by the solution casting method. First, 5% (w/v) solutions of PPDO and PDPA in chloroform were prepared, and then 10 mL of the solutions were put onto glass plates. The glass plates were exposed to an infrared lamp to rapidly evaporate the chloroform to yield the polymer films. The films were cut into 5 cm × 1.5 cm pieces for contact angle tests and 1 cm × 1 cm pieces for hydrolysis tests. Hydrolysis experiments of PPDO and PDPA were conducted in a 0.05*M* buffer solution (based on Na_2HPO_4 and KH_2PO_4) with an initial pH of 7.40 at 37°C. At each time point, samples were removed from the hydrolysis medium and dried until they achieved a constant weight at room temperature. The degree of hydrolysis was followed by the determination of M_w by GPC during hydrolytic degradation.

Solubility of PPDO and PDPA

Solutions of 0.4 g neat PPDO and PDPA with different phenylalanine content in 5 mL chloroform were stirred for 30 min at room temperature. The images of the solutions of the three systems were recorded using a Panasonic DMC-FS4 camera and were used to examine the solubility.

Cell Culture

Circular membranes with a diameter of 15 mm were excised from electrospun sheets of PDPA and PPDO and then sterilized with epoxy ethane. These membranes were wetted before being placed in a 24-well plate. The L929 mouse fibroblasts (2.5×10^4) were seeded onto the membranes. The cells were incubated in Dulbecco's modified eagle medium (DMEM, Gibco) supplemented with 10% fetal calf serum, and 100 U/mL penicillin and streptomycin in an incubator containing 5% CO_2 at 37°C. After 1, 3, and 5 days, membrane-adsorbed cells were harvested for MTT assay.

Fluorescent Staining and Microscopy Analysis

The membrane-adsorbed cells accompanied with the membranes were removed from the culture medium and stained with acridine orange (Fluka) and ethidium bromide (Fluka). After 20 min, the cells were observed under a fluorescence microscope (100 × amplification, DM IL, Leica Microsystems, Germany) after being washed with phosphate buffered saline (PBS). Under the microscope, living cells were clearly detected on the membrane with bright green cell nuclei; dead cells were stained red.

RESULTS AND DISCUSSION

Synthesis of PDPA

Scheme 1 shows the synthetic route of PDPA. Poly(*p*-dioxanone-*co*-L-phenylalanine) was prepared by ring opening copolymerization of PDO and L-Phe-NCA using $Sn(Oct)_2$ as catalyst. In the ^{1}H NMR spectrum, phenyl protons, methine protons, and methylene protons of L-Phenylalanine structure units appeared at 7.22, 4.58, and 3.05 ppm. In addition, compared to that of L-Phenylalanine *N*-Carboxyanhydride monomers, the amide protons in copolymer has shifted to 8.00 ppm which indicate the successfully polymerization of the monomers. With the reaction conditions mentioned above, the copolymers of PDO and L-Phe-NCA could be obtained assuredly according to our previous work.[34] In the ^{1}H NMR spectrum, peaks corresponding to methylene protons of *p*-(dioxanone) structure units appeared at 4.20, 4.16, and 3.69 ppm respectively. The integration of phenyl protons was used to confine the molar fraction of L-Phenylalanine structure units in the copolymer. The average molecular weights of polymers were obtained by GPC. The molecular weights and compositions of PPDO and PDPA are summarized in Table I.

Crystallinity of PDPA

Copolymerization is commonly used to change the crystallinity of polymers. Therefore, the crystallinity of PPDO and PDPA

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Table I. Molecular Weights, Compositions, Static Water Contact Angles, and Roughness Values of PPDO and Copolymers

Copolymer	In-feed molar ratio of PDO/L-Phe-NCA	M_w (10^3 g/mol)	Molar fraction		Static water contact angle (°)	Roughness Value (nm)
			L-Phenylalanine	*p*-(Dioxanone)		
PPDO	-	29.89	0	1.00	74.23	54.8
PDPA-1	20	37.29	0.053	0.947	67.14	25.1
PDPA-2	10	32.56	0.114	0.886	70.87	17.2

was investigated. Figures 1 and 2 show the cooling and heating scans at 10°C/min of PPDO and PDPA after keeping them at 130°C for 3 min in order to erase their thermal history. The exothermic peaks in Figure 1 resulted from the crystallization processes and their positions depend on the L-phenylalanine contents. The average molecular weights of the three samples are all around 30,000 g/mol; therefore, the effect of molecular weight could be ignored. All the relevant enthalpies (ΔH_m and ΔH_c) and the glass transition and melting temperatures (T_g and T_m) evaluated from Figures 1 and 2, respectively, are listed in Table II. From Table II, we can see that the L-phenylalanine contents have a significant influence on the crystallization behavior of PDPAs. PPDO which does not contain L-phenylalanine has the highest crystallization enthalpy (ΔH_c). This is attributed to the strong driving force of crystallization of PPDO. The crystallization enthalpy decreased with the increase contents of L-phenylalanine in the copolymer as the crystallization enthalpy (ΔH_c) of PPDO is 60.18 J/g, PDPA-1 is 42.72 J/g, and PDPA-2 is 0 J/g, indicating that the crystallinity of PDPAs decreases with increasing L-phenylalanine content (0–11.4%). PDPA-1 and PDPA-2 do not crystallize fully during cooling scan and exhibit cold crystallization exothermal peaks in the subsequent heating scan (Figure 2). The cold crystallization enthalpy ($\Delta H_{c,1}$) of PDPA-1 is much smaller than that of PDPA-2, indicating that PDPA-2 which has the highest L-phenylalanine contents crystallized much more difficult than PDPA-1. The cold crystallization temperature ($T_{c,1}$) of PDPA-2 (50.43°C) is much higher than that of PDPA-1 (27.56°C) indicating that even in the heating scan, PDPA-2 could not crystallize easily. The cold crystallization phenomenon could not be observed in the heating scan of PPDO as the crystallization develops fully in the cooling scan. The melting enthalpy (ΔH_m) of PDPA-2 is much lower than those of PPDO and PDPA-1, which means that PDPA-2 experience much lower crystallinity than the other two in both cooling and heating scan.

It could be seen from the above that compared to PPDO, the crystallinity of PDPA decreased obviously, furthermore, the crystallinity of PDPA decreased with the L-phenylalanine contents increasing. The reason for the decreased crystallinity of PDPA may be that the rigid benzyl groups hamper the flexibility and regularity of the PPDO chains and confine the movement of the copolymer chains. The glass transition temperature of PDPA increased from −9.43°C to −4.42°C as L-phenylalanine content increased from 0 mol % to 11.4 mol %, providing further evidence of the decreased flexibility of the copolymer with increasing L-phenylalanine content. It should be noticed that the L-phenylalanine segments in PDPA were dispersed in the PPDO segments and could not crystallize on their own because of the much shorter length and much lower contents. Therefore, there were no other crystallization peaks for L-phenylalanine segments.

In order to prove the conclusion in development, the morphologies of PPDO and the PDPAs in the isothermal crystallization process from melt were observed by POM (Figure 3). From Figure 3, it can be seen that upon crystallization at 55°C for 6 min, there were many completed spherocrystals of PPDO [Figure 3(a)], few scattered small crystals of PDPA-1 containing

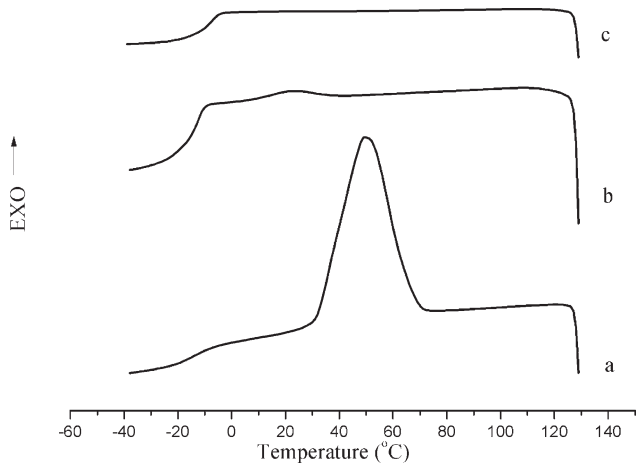

Figure 1. The cooling curves of PPDO and PDPA from melted state after being eliminated all thermal histories with (a) PPDO; (b) PDPA-1; (c) PDPA-2.

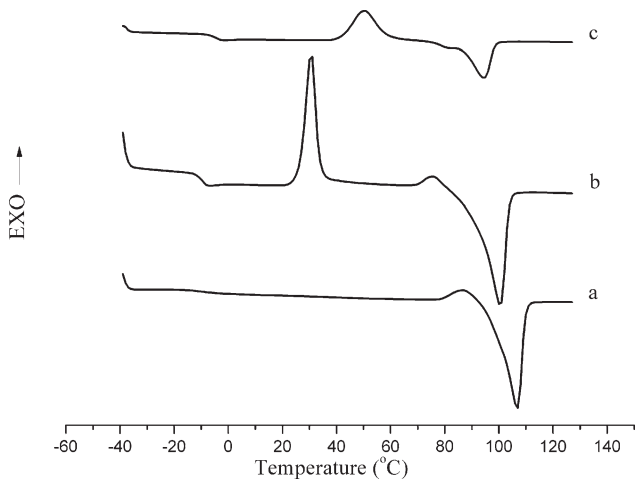

Figure 2. The heating curves of PPDO and PDPA after cooling scanning with (a) PPDO; (b) PDPA-1; (c) PDPA-2.

Table II. Relevant Transitions and Enthalpies Extracted from Figures 1 and 2

Sample	T_c (°C)	ΔH_c (J/g)	T_g (°C)	$T_{c,1}$ (°C)	$\Delta H_{c,1}$ (J/g)	T_m (°C)	ΔH_m (J/g)
PPDO	49.11	57.88	−9.43	-	-	106.73	69.04
PDPA-1	30.82	42.77	−7.18	27.56	4.77	99.42	64.99
PDPA-2	-	-	−4.42	50.43	27.61	94.50	27.39

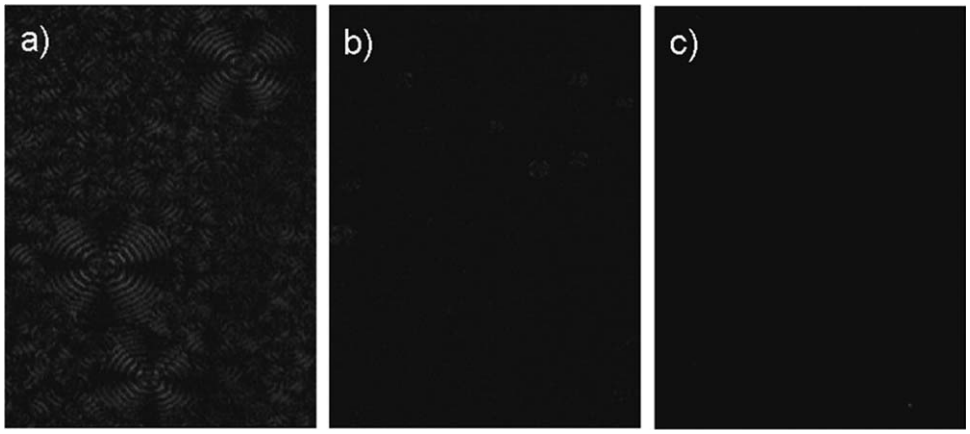

Figure 3. POM images of isothermal crystallization at 55°C from melted state after being eliminated all thermal histories with (a) PPDO; (b) PDPA-1; (c) PDPA-2.

5.3 mol % L-phenylalanine [Figure 3(b)], and no crystals of PDPA-2 containing 11.4 mol % L-phenylalanine [Figure 3(c)], indicating that the crystallization processes were inhibited as the L-phenylalanine content increased. This result is in accordance with the DSC investigation.

Solubility of PDPA

The dissolution of equal quantities of PPDO and PDPA in 5 mL chloroform after stirring for 30 min at room temperature is shown in Figure 4. The PPDO did not completely dissolve in chloroform, and formed a turbid mixture. In contrast, the PDPAs containing 5.3 and 11.4 mol % phenylalanine dissolved entirely in the chloroform to give clear solutions. It can be concluded that the solubility of PDPAs in chloroform is better than that of PPDO. It is very difficult for solvent molecules to penetrate crystalline polymer chains owing to their regular arrangement and strong intermolecular interactions. Thus, crystalline polymers are more difficult to dissolve in solvent than amorphous ones. Compared to PPDO, PDPAs had much lower crystallinity, which resulted in better solubility.

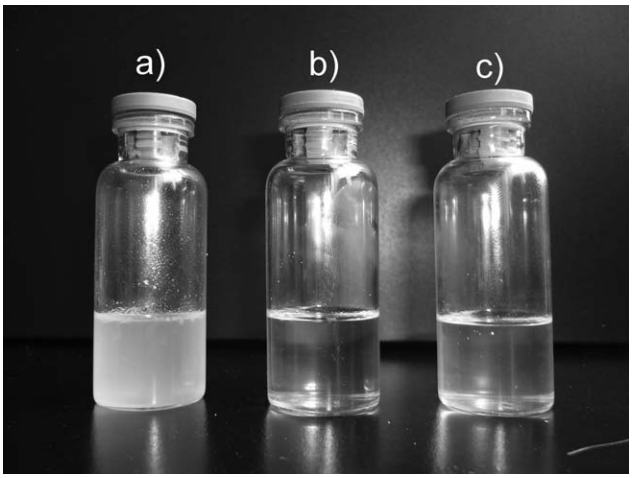

Figure 4. Photographs of polymer solution in chloroform at room temperature with (a) PPDO; (b) PDPA-1; (c) PDPA-2.

Hydrophilicity of PDPA

The results of static water contact angle testing of PPDO and PDPAs with different L-phenylalanine content are given in Table I. PPDO has the highest water contact angle value around 74.23° means that it is the most hydrophobic one of the three samples. Compared to PPDO, the water contact angle values of PDPAs are smaller, which means they are more hydrophilic. This phenomenon may result from the introduction of the more hydrophilic amide bonds in the copolymer.

However, compare the water contact angle value of PDPA-1 around 67.14° to that of PDPA-2 around 70.87°, it is clear seen that the water contact values does not increase with the L-phenylalanine contents. The difference surface topography of the membranes of those two samples may be the possible reason. In order to monitor the surface structures of membranes in more detail, PPDO, PDPA-1, and PDPA-2 membranes were analyzed by atomic force microscopy (AFM). AFM images of PPDO, PDPA-1, and PDPA-2 are given in Figure 5(a–c), respectively, and the roughness values of the three samples were summarized in Table I. It can be seen from Figure 5 that the surface of PPDO membrane is roughest (roughness value equal to 54.8

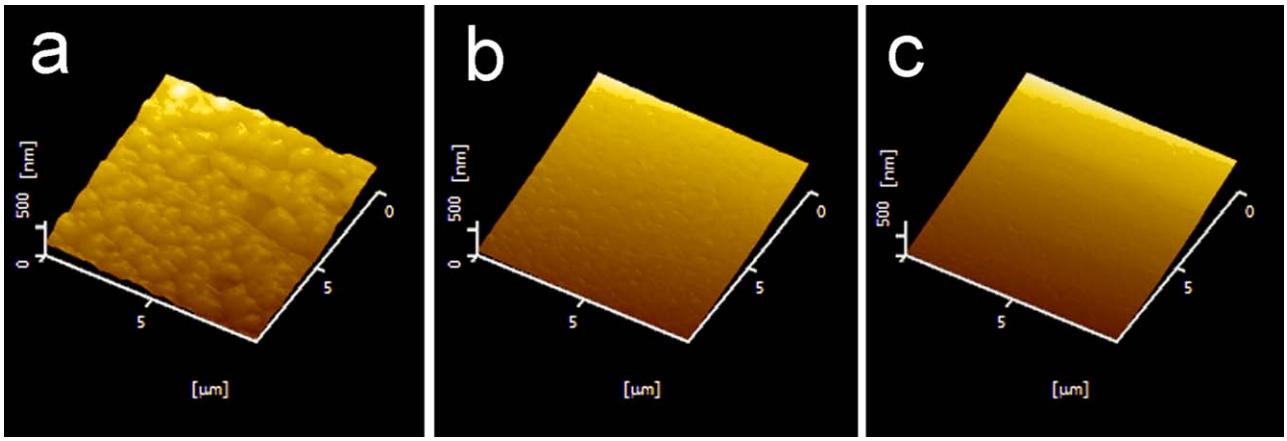

Figure 5. AFM images of surface topography of (a) PPDO, (b) PDPA-1, and (c) PDPA-2 membranes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nm) with many spherocrystals and that of PDPA-2 is flattest (roughness value equal to 17.2 nm) with not obvious crystal structure. AFM images are in good correlation with the results of DSC analysis. Depending on the crystallinity, surface topography, and roughness vary significantly and this results in the formation of surfaces with different hydrophilic characteristics. According to the Wenzel approach, water droplet wets the grooves of the material and this model predicts the effects of roughness of a surface on its wettability. If a material has a water contact angle greater than 90°, surface becomes more hydrophobic with increasing roughness. For materials exhibiting contact angles lower than 90°, increasing roughness yields more hydrophilic surfaces.[35,36] As a result, compared to PDPA-1, lower crystallinity of PDPA-2 induced flatter and less hydrophilic surface and then larger static water contact angle.

In Vitro Hydrolysis of PDPA

The changes in M_w with time during the hydrolysis process are shown in Figure 6. In the first 3 days, the losses of molecular weights of PDPA-1 are more than that of PDPA-2 and PPDO which may be attributed to its better hydrophilicity. However, in the subsequent stage after 3 days when all three types of polymers were adequately infiltrated by water, the degrees of hydrolysis of the copolymers became lower than that of PPDO. The reason for this may be that the amide bonds have better hydrolytic stability compared to ester bonds. These results demonstrate that the hydrophilicity and chemical structure were two

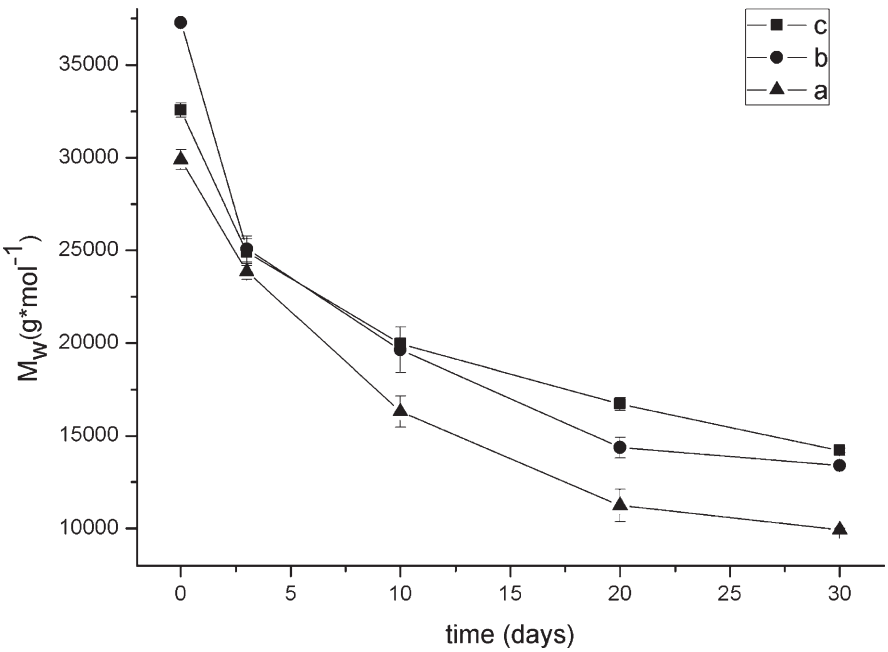

Figure 6. Weight average molecular weights changes during *in vitro* hydrolysis with (a) PPDO; (b) PDPA-1; (c) PDPA-2.

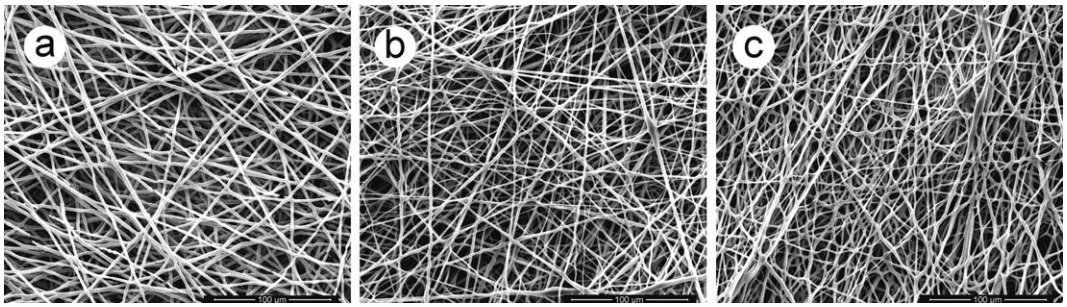

Figure 7. SEM images of electrospun membranes of (a) PPDO, (b) PDPA-1, and (c) PDPA-2.

influential factors of the *in vitro* hydrolysis of PDPA and PPDO. At the initial time, all the PPDO and PDPAs could not be infiltrated entirely, so the hydrophilicity was the primary factor, and then the M_w of PDPAs with more hydrophilicity decreased more quickly than PPDO. At the anaphase, the chemical structure played an important factor. The amide bonds from L-phenylalanine segments of PDPA blocked the hydrolysis of molecular chains, so the M_w of PDPA decreases more slowly than PPDO.

Cell Growth on PDPA Electrospun Membranes

Because the morphology of the electrospun membranes may affect the cell adhesion and proliferation, all membranes of the three samples were observed by SEM (Figure 7). There are not obvious differences of the membranes morphology including fiber diameters and pore diameters of the three samples. In addition, because the wettability of the electrospun membranes may affect cell proliferation, all membranes were wetted thoroughly with culture medium. The growth of L929 cells that adhered to the electrospun membranes of PPDO and PDPA culture can be judged from the fluorescent micrographs shown in Figure 8. No red dead cells could be seen in the visual fields of both PPDO and PDPA membranes. Some of the cells grew into the holes of the membranes or the gaps of nanofibers.

Figure 9 shows the cell MTT viability of the L929 cells on control, PPDO membrane, and PDPA membrane. In order to investigate the cell proliferation in detail, a parameter called relative cell proliferation rate (P%) was induced. $P\% = (OD_e/OD_c) \times 100\%$, where OD_e is the OD value of experimental

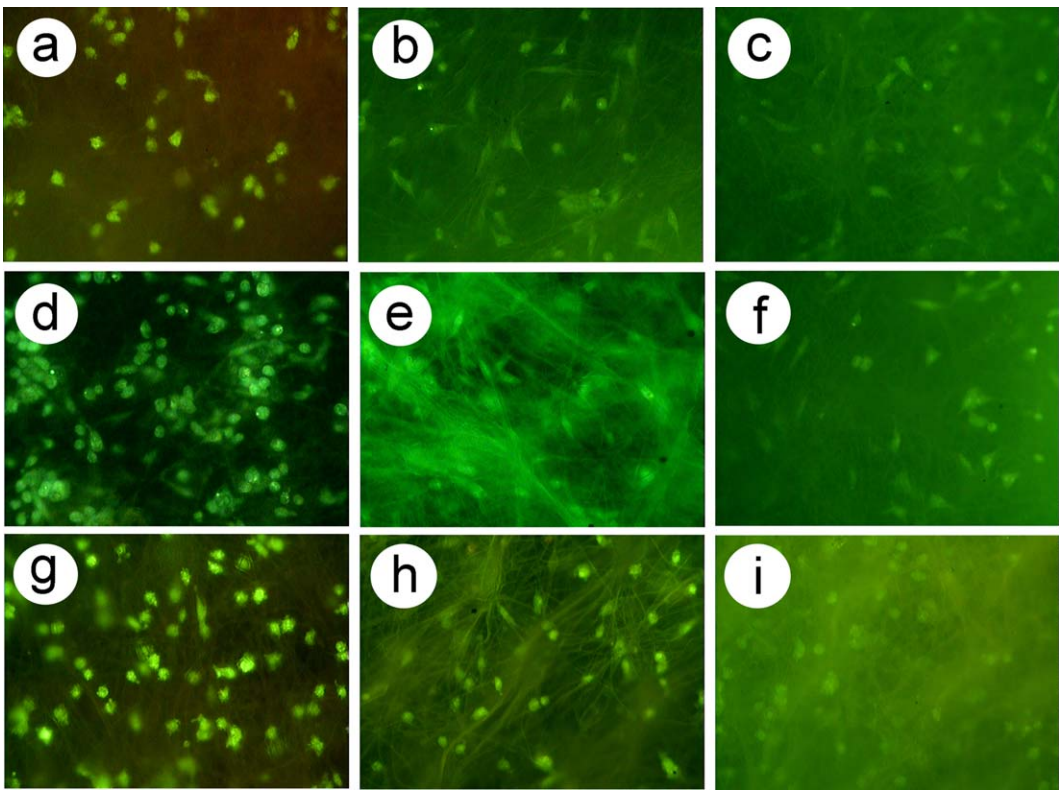

Figure 8. Fluorescent micrographs of L929 mouse fibroblasts culture on poly (PDO-co-Phe) electrospun membranes with (a) PPDO, (b) PDPA-1, and (c) PDPA-2 for 1 day; (d) PPDO, (e) PDPA-1, and (f) PDPA-2 for 3 days; (g) PPDO, (h) PDPA-1, and (i) PDPA-2 for 5 days. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

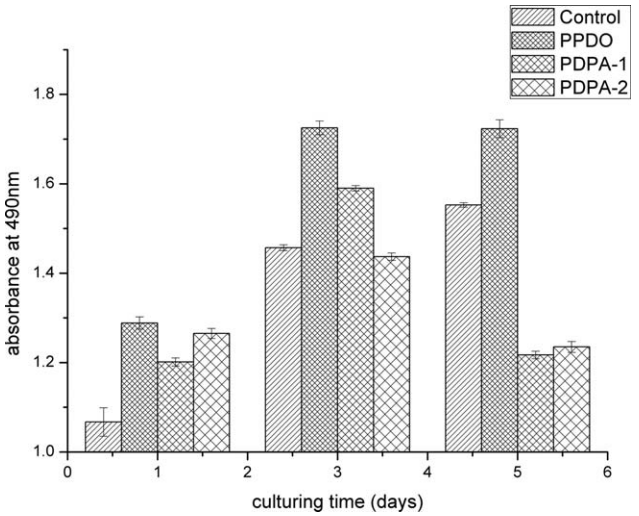

Figure 9. L929 mouse fibroblasts cell viability as measured by MTT absorbance at 490 nm after 1, 3, and 5 days of culture on electrospun membranes.

samples in MTT assay and OD_c is the OD value of control. The membrane whose P% is smaller than 100% would be a negative one for cell proliferation. The proliferation of L929 cells on PDPA membranes (P% of PDPA-1 equal to 112.6%, P% of PDPA-2 equal to 118.6%) was slightly inhibited compared to the PPDO membrane (P% equal to 120.8%) but still enhanced compared to the control after 1 day of culture. The obvious inhibition on L929 cell proliferation of PDPA was observed after 3 days of culture. The P% of PDPA-2 dropped to 98.6% which is less than 100% indicating the cell proliferation of L929 cell on PDPA-2 membrane was inhabited effectively. Furthermore, P% of both PDPA-1 and PDPA-2 membranes dropped under 100% (78.4% of PDPA-1 and 79.5% of PDPA-2) after 5 days of culture but P% of PPDO was still 118.6%. All of these results demonstrate that the L929 cell proliferation on PDPA membranes was inhibited and the inhibition effect increase with the culture time. The reason may be that L-phenylalanine was released gradually during the hydrolysis process of PDPA membranes. The inhibition of L-phenylalanine on rat myocardial fibroblast and vascular smooth muscle cell proliferation were reported by other researchers.[30,31] They also revealed that L-phenylalanine could block the expression of some early response genes. However, the molecular mechanism whereby L-phenylalanine inhibits gene expression is unknown. They inferred that the site of action of L-phenylalanine could be located downstream of receptors or elsewhere at the level of cell membrane. In this respect, some researchers reported that L-phenylalanine might modulate calcium channel conductance.[37] Thus L-phenylalanine may play a role in the regulation in the cellular functions in cells other than vascular smooth muscle cells and myocardial fibroblast. It is thus possible that in cultured L929 mouse fibroblasts, L-phenylalanine which was released into the culture medium during the hydrolysis process of PDPA membranes behaves as a calcium channel blocker on L929 mouse fibroblasts growth. As the concentration of L-phenylalanine in culture medium increases with the hydrolysis degree of PDPA membranes, the inhibition increases with the culture time.

CONCLUSION

The PDPAs were successfully synthesized using PDO and L-phenylalanine *N*-Carboxyanhydride as copolymerization monomers. The structure of the copolymer was characterized using ^{1}H NMR spectra and FTIR from which characteristic peaks were easily identified. The crystallization properties of PPDO and PDPA with different L-phenylalanine contents were investigated using DSC and POM. The crystallinity of PDPA decreased as the L-phenylalanine content increased. This is because the rigid phenyl groups of phenylalanine interrupted the regularities of the chains, thus confining their movement. The solubility of PDPA in chloroform was better than that of PPDO owing to the lower crystallinity of PDPA. In addition, the PDPAs were more hydrophilic than the PPDO since they contained more hydrophilic amide groups. The amide groups in the PDPAs also made them more stable toward *in vitro* hydrolysis than PPDO since the amide bonds are more difficult to hydrolyze than ester bonds. Finally, the L929 mouse fibroblasts proliferation on the PDPA electrospun membranes was inhibited effectively, indicating that PDPA has potential application in the areas where cell proliferation need to be inhabited such as adhesion prevention. Obviously, further experiments are needed to increase the L-phenylalanine contents and average molecular weights of PDPA. In addition, further investigations to clarify the mechanism of inhibition of L-phenylalanine on cell proliferation are necessary.

ACKNOWLEDGMENTS

This study was supported financially by the "Western Light" joint researcher program of Chinese academy of science.

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