Preparation and Characterization of Biocompatible Poly(L-lactic acid)/Gelatin Blend Membrane

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ABSTRACT: Poly(L-lactic acid) (PLLA)/gelatin blend membranes were prepared by solution casting method and using dimethyl sulfoxide (DMSO) as a cosolvent. Scanning electron microscopy measurement indicated that when the amounts of gelatin in PLLA/gelatin blend membranes are less than 5%, the sizes of phase separation of gelatin are in range of several hundred nanometers. These benefit in retaining the strength of PLLA membranes. X-ray diffraction analysis revealed that the semicrystalline PLLA became amorphous and the melt temperature of crystalline PLLA changes from 56 to 38°C after it was processed in DMSO solvent. When the content of gelatin is less than 5%, the films not only retain a good mechanical property but also improve the hydrophilicity of PLLA. The molecular motion of PLLA in blend films were also investigated by solid state ^{13}C CP/MAS NMR. On the basis of the result of relaxation times, it was found that the molecular motion of PLLA100 and PLLA/gelatin blends increased when compared with that of original PLLA. It was further verified that semicrystalline PLLA became amorphous. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 269–276, 2006

Key words: PLLA; gelatin; blend; solid state ^{13}C CP/MAS NMR; DSC

INTRODUCTION

Poly(lactic acid) (PLA) has been widely used in tissue engineering field because of its biocompatibility, biodegradability, and good mechanical properties.[1–3] PLA is produced by ring-opened polymerization of lactides or polymerization of lactic acid monomers. Generally, PLA is a copolymer of poly(L-lactic acid) (PLLA) and poly(D-lactic acid) (PDLA), which is originated from L-lactic acid and D-lactic acid, respectively.[4–6] PLLA is a semicrystalline polymer and PDLA is an amorphous polymer. Their biological, mechanical, and biodegradable properties are dependent on the distribution of chiral repeat unit, stereoregularity, and crystallinity.

The hydrophobicity of PLLA hinders the cell attachment and proliferation. This has a negative effect for its biomedical application. The approaches to enhance its hydrophilicity are appropriate modification, such as copolymerization with polylysine,[7] surface modification,[8,9] and blending with hydrophilic polymers. There are a lot of reports on PLA blends, such as blending with poly(ε-caprolactone),[10,11] poly(hydroxy butyrate),[12,13] poly(vinyl acetate),[14] and thermoplastic starch.[15] But up to now, just a few articles about blending PLA and gelatin were reported.

Gelatin is also widely used as biomaterial in tissue engineering and it is nontoxic, hydrophilic, and cell adhesive. The disadvantage of gelatin is that its mechanical strength is not enough when it is used as biomaterial. The aim of this study is to get a new kind of biomaterials, which have better mechanical strength and cell adhesive ability, by blending gelatin and PLLA. However, the polarity of PLLA is significantly different from that of gelatin; when blending them with screw extruder, a distinct phase separation will occur, which will deteriorate the mechanical property of PLLA, and on the other hand, the gelatin is easily denatured in the higher extruded temperature. Both PLLA and gelatin can be dissolved in dimethyl sulfoxide (DMSO), and so dissolving them and casting their mixed solution by using a mold is a potential method to get the target materials. In this study, PLLA/gelatin blend membranes with different ratios of two ingredients were prepared by solution casting method using DMSO as a cosolvent. The morphology and crystallinity of PLLA/gelatin blend films were investigated by scanning electron microscopy (SEM) and X-ray diffraction, respectively. Molecular motion of PLLA/gelatin blends was also studied by ^{13}C crosspolarization/magnetic-angle spinning solid-state (CP/MAS) NMR.

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EXPERIMENT

Materials

PLLA (98% L-lactide and 2% D-Lactide) was kindly provided by the Department of Chemistry Engineering, University of Massachusetts, USA. The average molecular weight determined by gel permeation chromatography (GPC) was 10^5. Bovine gelatin ($M_w = 1.5 \times 10^5$, $M_n = 10^5$ g) was purchased from Sigma company. Other reagents are of analytical grade.

Preparation of PLLA/gelatin blend membrane

The desired amount of PLLA and gelatin were dissolved in DMSO to form 5 wt % solution and then they were mixed in the ratios of PLLA/gelatin 100:0, 99:1, 98:2, 95:5, 90:10, 80:20, 50:50, 20:80, 10:90, and 0:100. Based on the amount of PLLA, the samples were coded as PLLA100, PLLA99, PLLA98, PLLA95, PLLA90, PLLA80, PLLA50, PLLA20, PLLA10, and PLLA0. Raw PLLA without DMSO treatment was marked as PLLA.

First, the mixtures of PLLA and gelatin with different ratios were magnetically stirred at 50°C for 30 min and then kept at room temperature overnight. Although the polarities of PLA and gelatin were different, they still can be dissolved in DMSO simultaneously. Second, the initial mixtures were further dispersed by using ultrasonic for 30 min, and then equal amount of each mixture was taken into equal plastic mold. All the molds were dried in an oven at 50°C under nitrogen atmosphere for a week. After this step, most of DMSO was evaporated and the original blend films were formed. Lastly, the blend films were transferred into a vacuum oven, and were further dried at 50°C for one week to remove DMSO completely until the weight of films was constant.

Thermal analysis

Differential scanning calorimetry (DSC) was conducted on Netzsch DSC 204 instrument and was calibrated by using indium as standards. All the experiments were performed in nitrogen atmosphere. Sample weights were 10–15 mg, and heating rate was $10°C/min^{-1}$.

X-ray diffraction

Wide-angle X-ray diffraction (Rigaku D/Max-Ra) experiment was performed using Ni-filtered Cu Kα (wavelength 1.54 Å) radiation detected by a scintillation counter with single-channel discrimination.

^{13}C CP/MAS NMR

Solid-state ^{13}C CP/MAS NMR was carried out on Varian Unity Plus 400 spectrometer at 100.6 MHz at 25°C. Spinning rate was kept at 4.5 kHz, the 90° plus was 5.5 μs, and the relaxation delay time was 5 s. Scans (1000) were performed for each sample. The inversion–recovery pulse sequence was used to measure spin-lattice time (T_1).

Contact angle measurements

The water contact angles on the surface of PLA/gelatin blend membranes were measured by putting a droplet of distilled water on the surface of each film at room temperature. For each specimen, the measurements were repeated for five times and average values were calculated.

Morphological analysis

SEM observation of PLLA/gelatin blend membranes was performed on a Philips SEM15 scanning electron microscopy. Samples were coated with gold prior to SEM observation.

Tensile testing

Tensile tests of PLLA and all blend membranes were performed on an Instron machine (4130 model) equipped with a 100 N cell. Each sample was cut into a length of 40 mm, an extent of 20 mm, and a thickness of 0.5 mm. All the tests were conducted at room temperature, with a deformation rate of 10 mm/min.

RESULTS AND DISCUSSION

Morphology of PLLA/gelatin blends

PLLA and gelatin have different polarity, and while blending them, the phase separation always exists in the sample no matter whatever methods are been used. The degree of phase separation will affect the mechanical strength of blend membrane. The phase distribution of blend membrane is observed using SEM. Figure 1 is SEM micrographs of surface of PLLA/gelatin membranes with various ratios. It is found that the phase morphologies of PLLA/gelatin blends change significantly along with the increase in contents of gelatin. It is noted that for PLLA100 [Fig. 1(a)], the darker area in the middle of micrograph is resulted from local overheat caused by high voltage electron bombardment, which exceeds the glass transition temperature (T_g) of PLLA, and making the sample distorted. Thus, the focus of microscope is altered. Figure 1(b) displays the SEM micrograph of PLLA99, where the white parts are gelatin phase domains and the dark ones are assigned to PLLA phase. It is obvious that the phase separation still occurs even at low content of gelatin. Gelatin phase domains are near globule with the size of several hundred nanometers.

When gelatin contents are 2% [Fig. 1(c)] and 5% [Fig. 1(d)], the morphologies remain nearly unchanged compared with that of PLLA99. The size of domains is also in the range of several hundred nanometers. As the content of gelatin is raised to 10%, the gelatin granules occur to aggregate and the size of granules increases to 1–4 μm. Furthermore, the phase morphology becomes rather abnormal. While the content of gelatin is increased to 20% [Fig. 1(f)], the distribution of gelatin granules appears in homogenous. The size of larger granules is about several microns and the size of smaller granules is several hundred nanometers. Nevertheless, in this case, gelatin is still a dispersed phase, whereas PLLA remains a continuous phase. When the content of gelatin is 50% [Fig. 1(g)], it is difficult to distinguish the two phases because of the ambiguous phase interface. With further increase of gelatin content up to 80% [Fig. 1(h)], an evident phase variation occurs. The gelatin phase becomes a continuous phase and forms beehive-like network structure; PLLA phase is dispersed. Some circular patterns with diameter of 50 μm appear when the content of gelatin is 90% [Fig. 1(i)]. PLLA is merely dispersed within the circular patterns where both PLLA and gelatin are separation phase. Outside is all the gelatin phase.

The degree of phase separation of gelatin in blend membrane has significant effect on the strength of blend membranes. Therefore, it is necessary to decrease the phase separation to avoid excessive loss of strength of blend membranes. On the basis of SEM micrographs, it is found that when the amount of gelatin in membranes is less than 5%, the sizes of gelatin granule is several hundred nanometers, and the small phase separation will be beneficial for the membranes to retain better mechanical strength. Further tensile test will be carried out.

X-ray diffraction

The crystalline state of PLLA also has an obvious influence on the mechanical performance of membranes. The crystallinity of PLLA and various PLLA/gelatin blends is checked by X-ray diffraction (Fig. 2). For PLLA, the diffraction peaks at 2θ of 16.8° and 19.5° are observed, reflecting its semicrystalline behavior. While for other samples, only board peaks are observed, i.e. the samples are amorphous. It is noteworthy that even for PLLA100, no crystal is formed, indicating the ordered stacking structure of PLLA is destroyed after DMSO proceeding.

We think that the destruction of crystalline structure of PLLA is attributed to the rearrangement of chain segments in the course of DMSO evaporation rather than to the addition of gelatin, since PLLA still remains semicrystalline after it is processed with acetonitrile.[16] This result is in agreement with SEM analysis. As aforementioned, the phase separation exists at all blend membranes, and no interaction in molecular scale occurs.

Mechanical properties of PLA/gelatin blends

The mechanical properties of PLLA/gelatin blends with varied ratios are tested, as shown in Figure 3. The elongations at break of all blend films are lower than 5%, indicating the films are brittle at room temperature. The tensile strength of PLLA/gelatin blends gradually decreases along with the increase of gelatin contents till 50%, at which a minimum appears. About 5%, the tensile strength of blends does not decrease a lot when compared with that of PLLA100. A reasonable explanation is that gelatin distributes homogenously in PLLA, and moreover the size of phase separation is in the range of several hundred nanometers [Fig. 1(b)–(d)], which is considered to have a little influence on the mechanical properties. The size of phase separation obviously increases upon increasing gelatin contents. Hence, the tensile strength drops markedly. This result suggests that to retain the tensile strength of PLLA, the contents of gelatin should be controlled in the range of 5%.

Contact angles of PLLA/gelatin blend films

Contact angle of water on the surface of biomaterials is useful parameter for the evaluation of biomaterial's hydrophilicity. Normal hydrophilic material has smaller contact angle and better cell adhesive. Gelatin is a hydrophilic biomacromolecule, but the contact angle of water on the pure gelatin film is unexpectedly 72°. Many other investigators[17,18] have pointed out that this phenomenon is due to the preferred orientation of hydrophobic moieties at the gelatin–air interface. Figure 4 shows the variation of contact angle as a function of gelatin content. It can be seen that there appears a minimum at 50% gelatin content, below which, the contact angles of blend films decreases with the increase of gelatin content. Contact angles of blend films are higher than that of pure gelatin when gelatin content is more than 5%. It implies that the incorporation of gelatin enhances the hydrophilicity of the films, and on the other hand, the preferred orientation of hydrophobic of gelatin is destroyed because of DMSO treatment, which results in the enrichment of hydrophilic moieties on the surface. As the gelatin content is further increased, the interactions between hydrophilic groups are dominant and consequently more of hydrophilic moieties tend to bury into the bulk of gelatin, generating higher contact angle. Gelatin content (5%) in blend film is suitable to get a material, which possesses better hydrophilicity than PLLA.

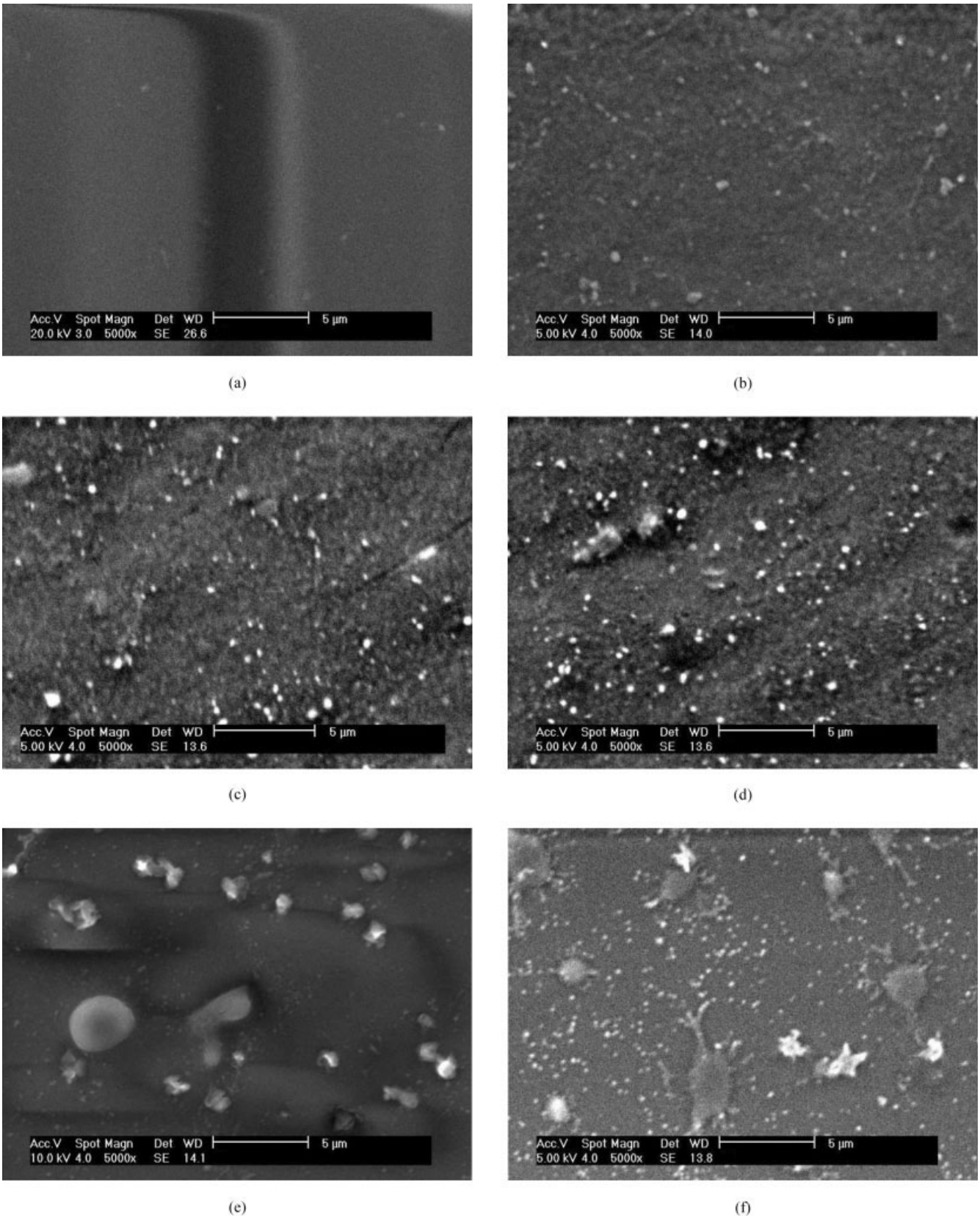

Figure 1 SEM micrographs of surface of PLLA/gelatin blends with various ratios: (a) PLLA100, (b) PLLA99, (c) PLLA98, (d) PLLA95, (e) PLLA90, (f) PLLA80, (g) PLLA50, (h) PLLA20, (i) PLLA10, and (j) PLLA0.

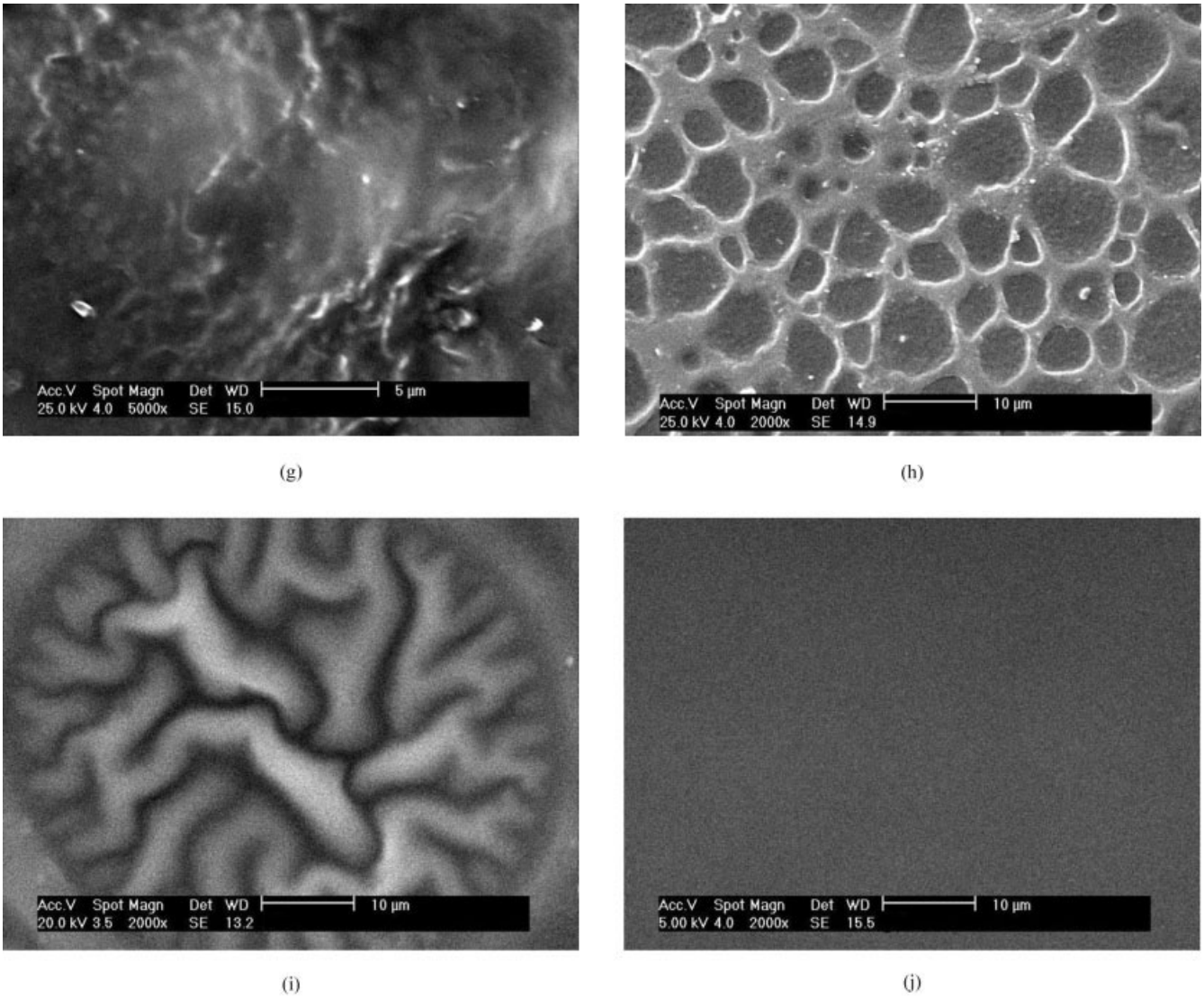

Figure 1 (*Continued from the previous page*)

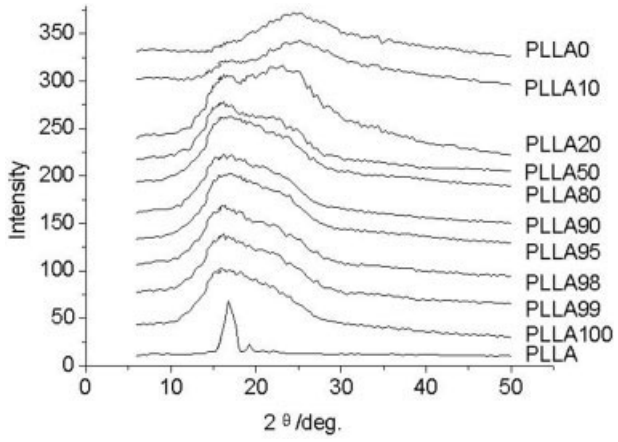

Figure 2 X-ray spectra of PLLA and various PLLA/gelatin blend membranes.

Thermal analysis

The thermal property of blend membrane also is important for its applications, and DSC thermograms of PLLA and PLLA/gelatin blend membranes are carried out as shown in Figure 5. The melt temperature of crystalline PLLA is 56°C. After PLLA is dissolved in DMSO and redried, a peak at 38°C appeared, and it should belong to the glass transition temperature (T_g) of PLLA sample, indicating semicrystalline PLLA becomes amorphous, which agrees well with the results of X-ray diffraction. T_g's of other blend membranes containing 1, 2, and 5% of gelatin are nearly constant. This means the amounts of gelatin in blend membranes do not affect the T_g of PLLA significantly, indicating they are not compatible in molecular scale. At 10%, T_g of blend film is not obvious. Apart from PLLA, a broad peak from 110 to 200°C on other four DSC curves is observed. This peak may be attributed

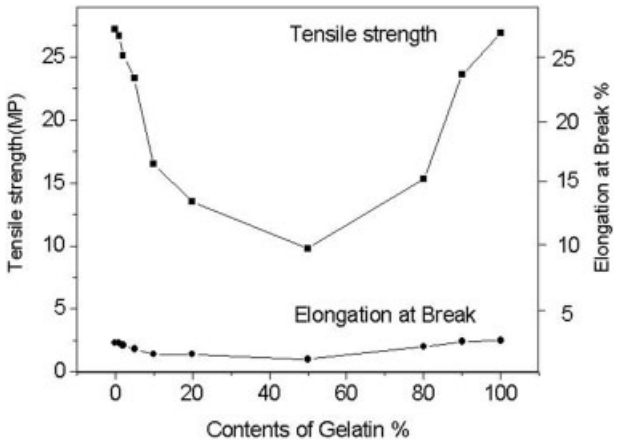

Figure 3 Mechanical properties of PLLA/gelatin blend membranes (at 25°C).

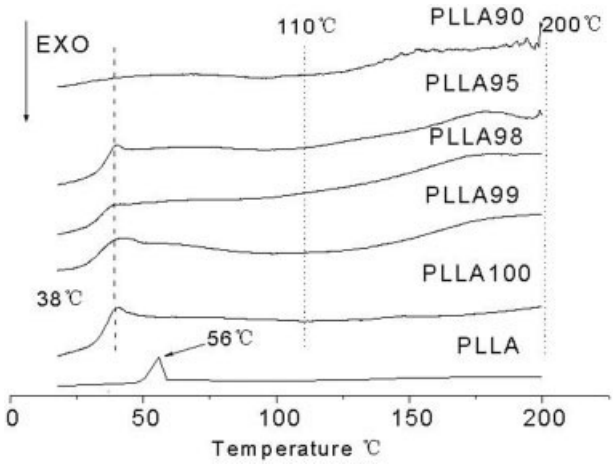

Figure 5 DSC thermograms of PLLA/gelatin blend membranes.

to the evaporation of remaining trace DMSO during DSC experiments. The decrease of T_g of PLLA is mainly originated from the formation of amorphous PLLA caused by DMSO processing. Because of just less than 10% of gelatin is added in blend membranes, the contribution of change of gelatin aggregate structure for this broad peak can be ignored.

^{13}C CP/MAS NMR spectroscopy

^{13}C CP/MAS NMR spectroscopy is used to study the structure and molecular motion of PLLA and PLLA/gelatin blend membranes. Taking into account both the mechanical properties of materials and intensity of NMR signal, we studied only those samples with gelatin content less than 10%. Figure 6 shows ^{13}C CP/MAS NMR spectra of PLLA and PLLA/Gelatin blends. The peaks of 17, 70, and 170 ppm are attributed to —CH_3, —CH, and —C═O, respectively. Note that the peaks in the range of 10–80 ppm are similar for all samples, not affected by gelatin. Moreover, the resonance at 170 ppm is slightly different, but cannot be distinguished. More sensitive parameter "spinning lattice relaxation time T_1"[19] is a potential way to find the difference between the samples. In this study, the relaxation times of samples are measured, and based on relaxation times the molecular motion states of PLLA are checked, and accordingly the stacking structure of PLLA is elucidated.

Figure 7 shows the stack NMR spectra of PLLA with various delaying times (only stack spectra of PLLA is displayed, other samples are analogous). One can see that the NMR signals decay as the delaying time is increased. The decay speeds for various functional groups are varied. The decay speed of three resonance peaks is methyl carbon > methylene carbon > ester carbon. Obviously, relaxation time of methyl carbon is the shortest and ester carbon is the longest. The intensity of resonance peak decays exponentially. Table I

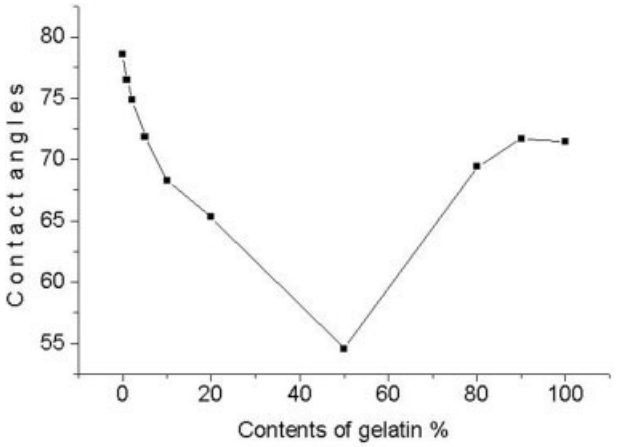

Figure 4 Contact angles of water on the surface of PLLA/gelatin blend films.

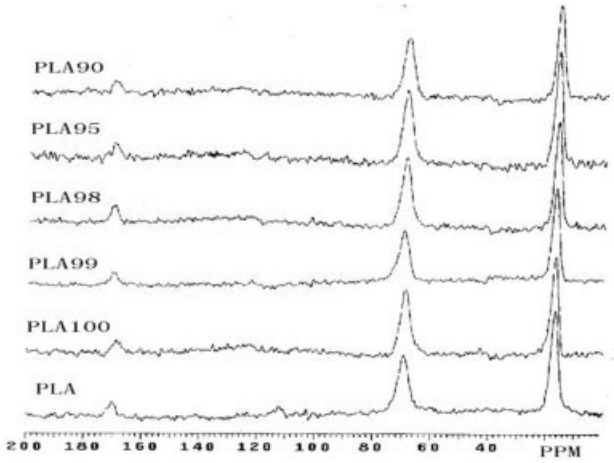

Figure 6 ^{13}C CP/MAS NMR spectra of PLLA and PLLA/gelatin blend membranes.

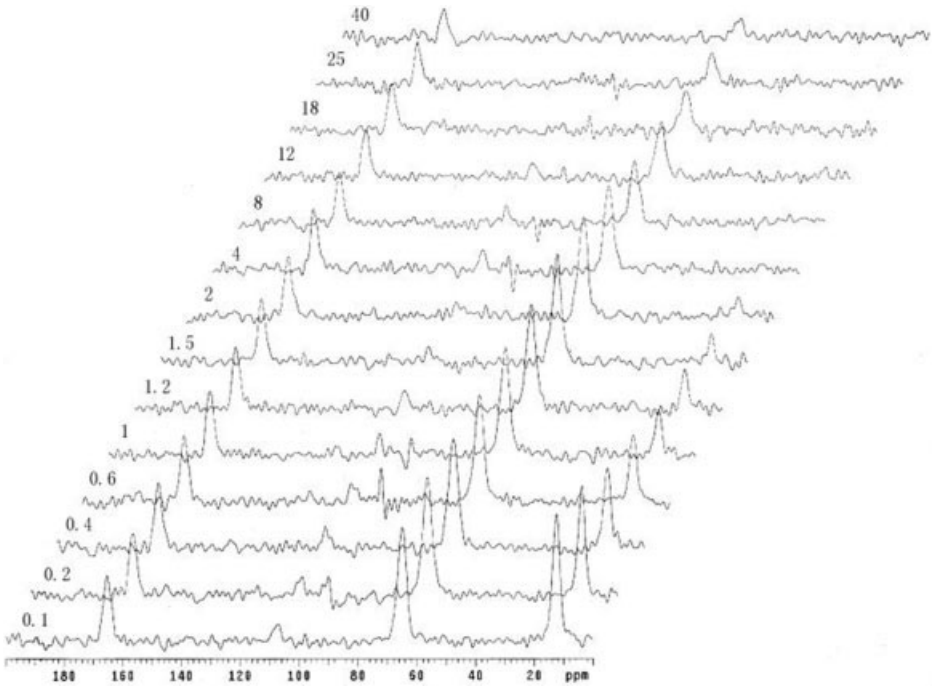

Figure 7 Stack NMR spectra of PLLA recorded at various delaying times (s).

lists T_1 of ^{13}C in various PLLA/gelatin blend samples. The relaxation time is related with the correlation times, which reflects molecular mobility. For semicrystalline polymers, especially for the T_g's of polymer that are above room temperature, the relaxation time is enhanced with the increase of correlation time, and correspondingly molecular mobility is lowered, for instance for PET, PBT, and PNT.[20–22] PLLA also belongs to this kind of material. T_1 values of unprocessed PLLA and PLLA/gelatin blends are listed in Table I. The relaxation times of PLLA are longer than those of other samples. The longer relaxation times indicate that the molecular mobility is decreased, which is presumably due to the hindrance of crystalline region of PLLA. After DMSO processing, i.e., for PLLA100, the relaxation times of three resonance peaks are lowered compared with those of PLLA, suggesting that molecular motion is enhanced. Thus, it is considered that semicrystalline PLLA has become amorphous. This is consistent with X-ray and DSC results. The relaxation times of three functional groups for other four samples remain almost unchanged compared with those of PLLA100. The incorporation of gelatin does not affect the molecular motion of PLLA100. It suggests that no interaction at molecular scale between gelatin and PLLA occurs. But it cannot be excluded that there might be the weak interaction between gelatin and PLLA at interface. This interaction cannot affect the molecular mobility of PLLA bulk.

TABLE I
Spin-Lattice Relaxation Times (T_1) of ^{13}C of PLLA in Various PLLA/Gelatin Samples

Samples	T_1 (s)		
	^{13}C—O	^{13}CH	$^{13}CH_3$
PLLA	37.3 ± 9.1	12.7 ± 1.0	0.86 ± 0.04
PLLA100	15.4 ± 3.1	7.9 ± 0.4	0.63 ± 0.02
PLLA99	17.6 ± 6.0	8.9 ± 0.7	0.58 ± 0.06
PLLA98	14.8 ± 4.2	9.2 ± 0.8	0.62 ± 0.05
PLLA95	12.6 ± 6.4	8.4 ± 0.7	0.65 ± 0.04
PLLA90	13.5 ± 5.7	8.2 ± 0.6	0.66 ± 0.06

CONCLUSIONS

PLLA/gelatin blend membranes are prepared by solution casting method. The blending films with the size of phase separation being in the range of several nanometers are obtained. When gelatin content is less than 5%, PLLA/gelatin blend films not only possess better mechanical property but also are more hydrophilic than PLLA. The crystalline PLLA is destroyed after DMSO processing. Semicrystalline form of PLLA converts into amorphous form. It seems that the breakage of crystalline is not influenced by the incorporation of gelatin. Relaxation times of blend films are measured by using ^{13}C CP/MAS NMR. On the basis of the result of relaxation times, it is found that molecular motions of PLLA100 and PLLA/gelatin blends are increased compared with that of PLLA. It is further verified that semicrystalline PLLA becomes amorphous.

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