Preparation and Characterization of Electrospun, Biodegradable Membranes

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ABSTRACT: Nonwoven, biodegradable membranes fabricated by electrospinning have recently attracted a great deal of attention for biomedical applications. In this study, microporous, nonwoven membranes of poly(L-lactide) and its copolymers and blends were fabricated through electrospinning. The structures and morphologies of the electrospun membranes were investigated with scanning electron microscopy, differential scanning calorimetry, and X-ray diffraction. Different polymer membranes, incorporated with carmofur, were fabricated, and their drug release profiles were investigated. Scanning electron microscopy images showed that the fiber diameters were down to the nanometer range. The diameters and morphologies of the nanofibers depended on processing parameters such as the solution properties (concentration and polymer molecular weight), applied electric voltage, solution feeding rate, and needle diameter. Differential scanning calorimetry showed that the crystallinity of the electrospun membranes was lower than that of the cast film. For all the membranes incorporated with the drug, there was a burst release in the first 10 h of incubation in phosphate-buffered saline at 37°C. Poly(glycolide-*co*-lactide) membranes showed faster and more complete drug release than poly(L-lactide), and this could be attributed to its faster degradation. The incorporation of polylactide–poly(ethylene glycol) could shorten the drug release time. A combination of suitable degradable biomaterials with an appropriate electrospinning process could be useful in the fabrication of a new kind of membrane suitable for different biomedical applications such as tissue engineering and drug delivery. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 3390–3397, 2008

Key words: biocompatibility; biodegradable; biofibers; biological applications of polymers; biomaterials

INTRODUCTION

Electrospinning has been recognized as an efficient technique for the fabrication of polymer nanofibers.[1] Various polymers[2–4] have been successfully electrospun into ultrafine fibers in recent years from solutions and melts. As we all know, when the diameters of polymer fiber materials decrease from micrometers to submicrometers or nanometers, some amusing characteristics appear, such as very large surface area to volume ratios, flexibility in surface functionalities, and superior mechanical performances in comparison with any other known forms of the materials.[5–7] These outstanding properties make polymer nanofibers optimal candidates for many important applications.[8] Considerable efforts have been made to develop scaffolds for tissue engineering over the last decade, using biodegradable and biocompatible synthetic or natural polymers. The design of scaffolds should principally mimic the structure and biological function of native extracellular matrix proteins,[9] which provide mechanical support and regulate cell activities.

Linear aliphatic polyesters such as polyglycolide, polylactide (PLA), and their random copolymer poly (glycolide-*co*-lactide) (PLGA) are often used as the base materials for implant devices, such as suture fibers and scaffolds for tissue engineering.[10,11] These materials meet several controlled release criteria; that is, they are biocompatible and biodegradable and can provide high efficiency for drug loading.[12] Many different techniques have been developed to fabricate nanostructured, biodegradable articles such as microspheres, foams, and films. It has been demonstrated that the molecular structure[13] and morphology of a polymer play a major role in the degradation and mechanical properties of the final products. The release profiles of entrapped drugs can be fine-tuned by the control of the degradation

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rate of the polymer matrix through the control of the molecular weight and molecular weight distribution, material composition, and porosity of the carrier.

Lee et al.[14] studied the relationship between the solution properties [e.g., conductivity, surface tension, viscosity, and dielectric constant of different poly(ε-caprolactone) solutions] and the morphologies of electrospun fibers. Li et al.[11] compared the chondrogenic activities of cultures of bone-marrow-derived mesenchymal stem cells (MSCs) seeded in nanofibrous poly(ε-caprolactone) scaffolds with those maintained as high-density cell pellet (CP) cultures. Zong et al.[15] studied the crystallinity of a PLGA (GA : LA = 90 : 10) membrane prepared by electrospinning.

Carmofur has antitumor effects in the human body. Some studies on the cytotoxic activity of carmofur against colorectal cancer cells have been reported, but the controlled and sustained delivery of carmofur has not been studied. In this study, carmofur was added to a polymer solution to form a nanofiber membrane, which could be used as a mechanical barrier based on nonwoven, nanofibrous, biodegradable membranes. The capability for the local delivery of anticancer antibiotics would increase their desired utility in biomedical applications, particularly in the prevention of postsurgical adhesion and infections.

In this study, the effects of experimental parameters[16] such as the electric field intensity and collecting distance on the morphologies of poly(L-lactide) (PLLA) nanofibers were investigated. Carmofur, a hydrophilic drug, was chosen as the model drug. The base materials for the electrospun, fibrous scaffolds were PLLA, PLGA (LA : GA = 85 : 15), and polylactide–poly(ethylene glycol) (PLA–PEG). The drug release profiles of electrospun membranes from the different polymers were investigated.

EXPERIMENTAL

Materials

Semicrystalline PLLA with a weight-average molecular weight of 1.4×10^5 g/mol and the copolymer PLGA (lactide/glycolide = 85:15) with a weight-average molecular weight of 1.3×10^5 g/mol were prepared through a ring-opening reaction from L-lactide and glycolide with Sn(Oct) as the catalyst. PLA–PEG was fabricated in our laboratory with a weight-average molecular weight of 6.0×10^4. Methylene chloride and dimethyl formamide (DMF) were purchased commercially.

Preparation of the polymer solution

A mixed solvent of methylene chloride and DMF with a typical weight ratio of 1.5 : 1.0 (methylene chloride/DMF by volume) was used to dissolve the semicrystalline PLLA. For PLGA, DMF was used as the solvent. For the drug release study, 8% carmofur (mass percentage of the drug to the polymer) was added to polymer solutions.

Electrospinning

In a typical electrospinning process, the polymer solution was supplied though a stainless steel needle with a diameter of 0.3 mm. One of the electrodes, connected to a high-voltage supply (BGG 6-313), was immersed in the polymer solution. The high-voltage supply was capable of generating a direct-current voltage up to 60 kV. The solution was continuously supplied at a rate of 5 mL/h. Different voltages, ranging from 10 to 30 kV, were applied for electrospinning. The distance between the needle tip and collector ranged from 10 to 25 cm.

Scanning electron microscopy (SEM)

The morphologies of the obtained nanofiber membranes were examined with SEM (S-2360N, Hitachi, Tokyo, Japan) after the membranes were gold-coated. The average diameters and diameter distributions were obtained with an image analyzer.

Differential scanning calorimetry (DSC)

DSC measurements were carried out with a TA Q100 (TA Instruments, New Castle, DE). An approximately 5-mg sample was hermetically sealed in an aluminum disc for these measurements. A typical temperature profile for the DSC study was 0–200°C at a rate of 10°C/min.

X-ray diffraction (XRD)

XRD of the polymer-cast film and nanofibrous membranes was carried out with a Rigaku D/max (Rigaku Company, Ltd., Tokyo, Japan) with copper as the target. The wavelength was 1.5406 Å. The scanning range was 5–70°.

Drug release profiles

The release of carmofur from the electrospun membranes was monitored with an ultraviolet–visible spectrophotometer (U-1800, Hitachi) at the wavelength of 265 nm. The membranes were incubated at 37°C in 500 mL of a phosphate buffer solution (pH 7.4) in a medicine dissolution instrument (ZRS-8G, Shantou, China). At a given time, 3 mL of the buffer was taken out, and an equal amount of fresh buffer was added to the incubation solution. The UV absorbance of carmofur in the buffer was detected and converted to the carmofur concentration according

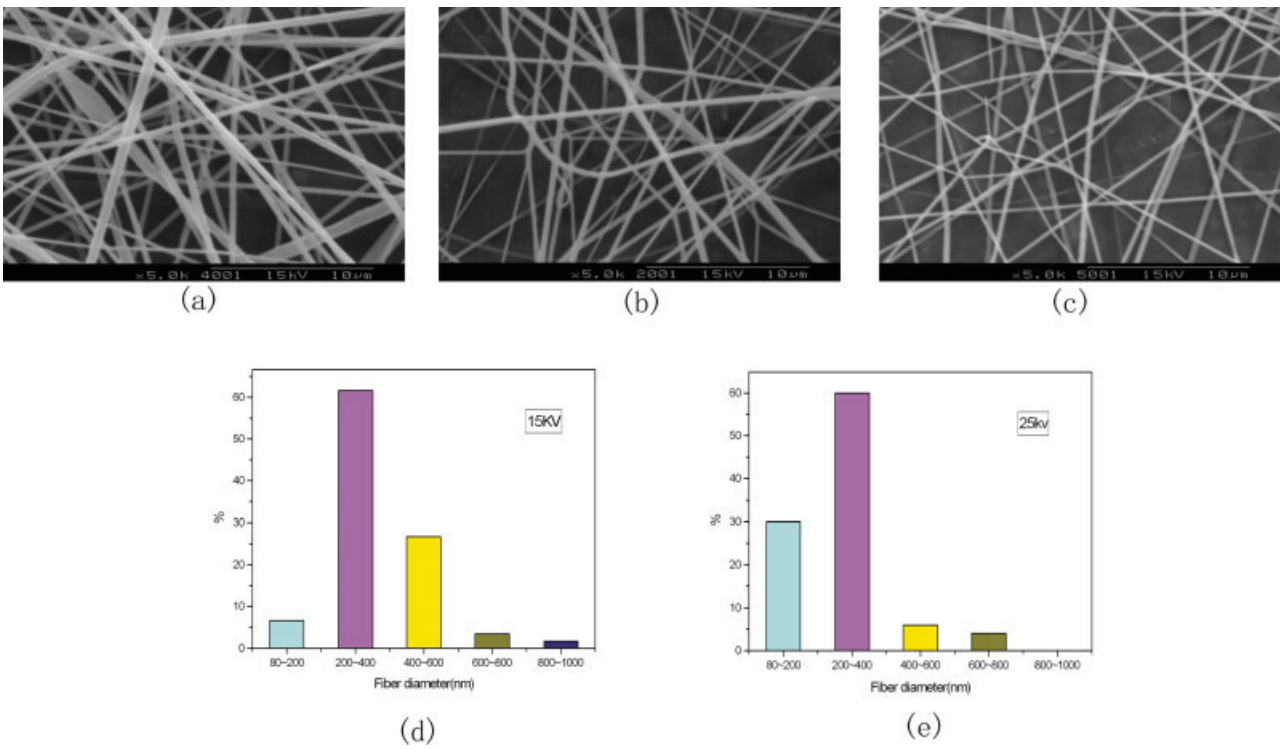

Figure 1 Effect of the electric field on the nanostructure of the fibers. The voltages were (a) 15, (b) 20, and (c) 25 kV, respectively. The diameters of the nanofibers were (d) 15 and (e) 25 kV. The PLLA concentration in methylene chloride + DMF mixed solvent in 8% and the feeding rate was 5 mL/h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to the calibration curve of carmofur in the same buffer. Then, the accumulated amount of the released carmofur was calculated as a function of the incubation time.

RESULTS AND DISCUSSION

Effect of the electric field

The shape of the initiating droplet at the end of the needle can be changed by the alteration of any of the processing parameters, including the applied voltage, the polymer concentration of the solution, and the distance between the collector and needle tip. In this study, it was confirmed that the morphologies of the nanofiber changed with an increase in the applied voltage from the typical cylindrical shape to a bead or string structure and then to a bead-free uniform nanostructure. The jet initiation is a self-accelerating process. Once the electric field is applied, the surface of the liquid at the tip of the needle becomes charged. When the electric field is high enough, the electric force overcomes the surface tension, and then a straight and electrically charged jet is ejected. Thus, the balance between the electric force and surface tension is critical for determining the initial shape of the polymer solution at the needle tip. In this study, when the voltage was below 14 kV, the droplet could not be carried away quickly. The droplet that gathered at the needle tip was larger than the hole diameter, so the jet was initiated from the suspended droplet but was elongated only to a hemispherical shape or strings of beads. With an increase in the applied voltage, the droplet that was suspended at the needle tip formed a Taylor cone and was carried away faster to form bead-free fibers. As shown in Figure 1, the diameters of the nanofiber decreased with the applied voltage increasing. When the applied voltage increased from 15 to 25 kV, the percentage of fibers ranging from 80 to 200 nm increased from 6 to 30%.

The main factor for the thinning of fibers from a micrometer scale to a nanometer scale is the whipping instability of the fluid, which causes bending and stretching of the jet. In this study, when the electrified jet fluid was accelerated and thinned along its trajectory, radial charge repulsion resulted in the splitting of the primary jet into multiple subjets in a process known as splaying. The final fiber size seemed to be mainly determined by the number of subsidiary jets that developed. The increasing voltage applied to the solution increased the electrostatic force between the multiple subjets, which induced extensive bending and stretching of the jets. Consequently, the obtained fibers had smaller diameters.

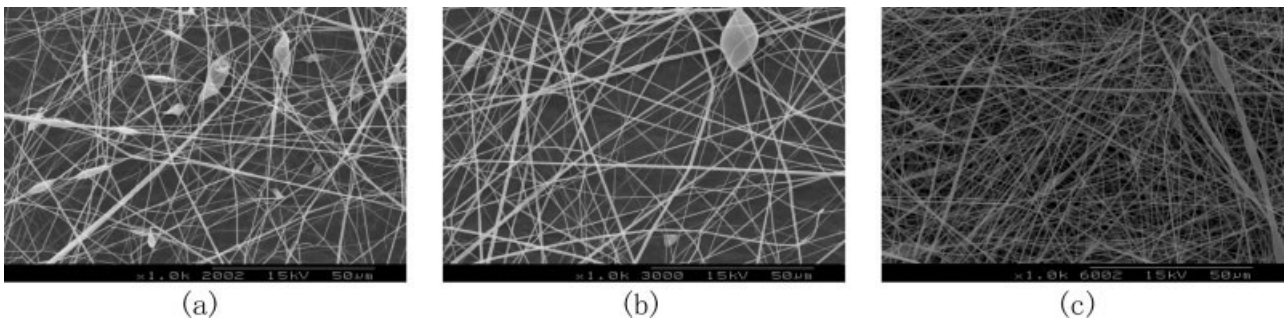

Figure 2 Effect of the gap distance on the nanostructure of the fiber. The distances were (a) 15, (b) 20, and (c) 25 cm. The PLLA concentration in methylene chloride + DMF mixed solvent is 8%, the feeding rate was 5 mL/h, and the applied voltage was 15 kV.

Effect of the distance between the needle tip and collector

The distance between the needle tip and collector is another parameter that affects the final nanofiber morphologies. During the electrospinning process, the jet mainly experiences bending and splitting, which are also the main contributions to the final nanodimension. It is easy to imagine that the longer the distance is, the greater the stretch ratio is. On the other hand, as the jet fluid driven by the electric forces is unstable during its trajectory toward the collecting screen, the multisubjets bend. The longer the distance is, the more obvious the effect is of bending on the fiber diameter, as shown in Figure 2. As we know from electrospinning theory, the formation of nanofibers is a complicated process of instability. In this study, the solvent evaporated, and the fiber solidified. When the distance between the tip needle and collector was 15 cm or even shorter, there was insufficient time for the solvent in the jet to evaporate in such a short distance to form uniform fibers. Instead, the remaining solvent on the collector damaged the morphologies of the fibers and led to the formation of beads. As the distance increased, complete evaporation of the solvent was in favor for fiber formation, so the beads gradually disappeared, and uniform nanofibers formed.

Effect of the solvent

The choice of the solvent plays an important role in the electrospinning process. For different polymers, different solvents are used to form nanofibers. The solution conductivity, surface tension, viscosity, and dielectric constant of the solvent should be taken into consideration. In this study, chloroform and a mixed solvent of methylene chloride and DMF (methylene chloride/DMF = 1.5/1) were chosen as the solvents for PLLA. Both solvents are capable of dissolving PLLA to form a uniform solution. However, as far as electrospinning was concerned, the two solvents exhibited completely different effects. As shown in Figure 3, when the mixed solvent was used, the fiber diameter was much smaller than that of the fiber obtained from the chloroform solution. The former was significantly smoother than the latter. A better understanding of this result can be obtained from the process of electrospinning. During the electrospinning process, a great change took place in this short time through bending, stretching,

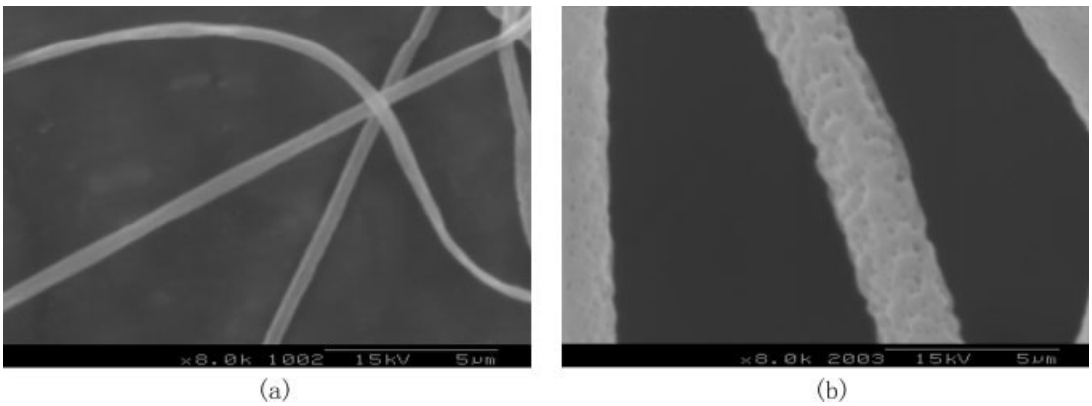

Figure 3 Effect of the solvent: (a) methylene chloride and DMF with a typical weight ratio of 1.5 (methylene chloride/DMF by volume) and (b) chloroform.

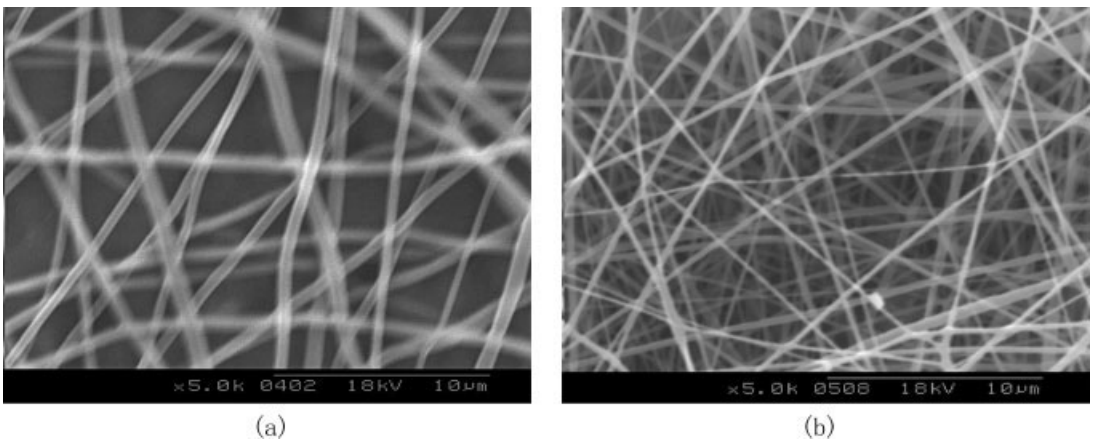

Figure 4 Effect of the incorporation of medicine: (a) the nanofiber without carmofur and (b) the PLLA nanofiber with carmofur.

and so forth. With the mixed solvent, the jet split into multijets. On the contrary, the chloroform solution was sprayed into jets without splitting along its trajectory; consequently, a thick fiber was formed, which might favor the formation of a superfine fiber with a core–shell structure to enwrap medicine. This might also be attributed to the solution conductivity and surface tension of the two solvents. As long as no splitting is involved, solvent evaporation seems to be much slower and to be encumbered by the thick fiber diameter, so a coarse surface on the fiber is formed when the solvent is evaporated.

Effect of the incorporation of medicine

As shown in Figure 4, the incorporation of carmofur had a remarkable effect on the morphologies of the nonwoven fibers. The diameter of the fibers decreased with the addition of carmofur. This phenomenon can be explained by the salt effect of carmofur.[18] Generally, the morphologies of electrospun nanofibers depend on solution parameters such as the conductivity, viscosity, and surface tension. When a drug is added to a polymer solution, the viscosity and surface tension of the mixed solution can be altered. Meanwhile, the conductivity of the solution can be increased by the presence of ionized drug molecules, which increase the charge intensity of the jet, resulting in the reduction of the fiber diameter.

Thermal behaviors of the electrospun fibers and cast film

Zong et al.[3] performed DSC and XRD analysis of PLLA nanofibers. They reported that the polymer chains were noncrystalline but highly oriented. However, in this study, as shown in Figure 5, the electrospun fiber had a lower glass-transition temperature (T_g) than the cast film. Compared with the polymer-cast film, the nanofibrous membrane had a higher ratio of the surface area to the volume, and the nanofibrous structure favored heat transformation, so T_g was lower for the nanofibrous membrane. At the same time, the nanofibers incorporated with carmofur had smaller fiber diameters and thus an even higher ratio of the surface area to the volume, so the mixed fiber membranes had the lowest T_g value.

During the electrospinning process, there was less time for the material to develop a highly ordered structure in comparison with the solution casting film. Therefore, the PLLA cast film demonstrated perfect crystallization. As shown in Figure 5, the melting temperature (T_m) and melting enthalpy (ΔH_m) of the nanofibrous membrane were lower than those of the cast film.

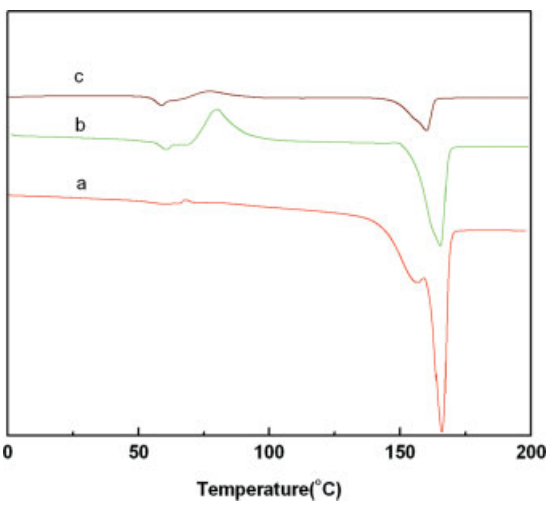

Figure 5 DSC curves of (a) the polymer-cast film, (b) the nanofibrous membrane, and (c) the nanofibrous membrane with carmofur. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Crystallinities of the PLLA Cast Film

Sample	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_c (J)	ΔH_m (J)	D_c (%)
Cast film	67.46		166.12		54.09	57.8
Electrospun fiber	60.01	79.82	165.55	19.23	40.23	22.4
Electrospun fiber incorporated with carmofur	58.19	76.99	160.37	10.08	33.99	25.5

During the electrospinning process, the fiber drops onto the collector in a short time not long enough for crystallization as a cast film. Therefore, when heated, the electrospun membrane recrystallizes at about 70°C. The degree of crystallinity (D_c) of three samples was calculated from the crystallization enthalpy (ΔH_c) and ΔH_m (ΔH_m of 100% crystallized PLLA was 93.6 J/g).[17] As shown in Table I, the crystallinities of the PLLA cast film, electrospun membranes, and membranes incorporated with carmofur were 57.8, 22.4, and 25.5%, respectively. These results are in agreement with the XRD results in Figure 6.

Crystalline structure of the electrospun membranes and cast film

As reported by other groups earlier, electrospinning retarded the crystallization process of the semicrystallized polymers. Similar behavior was also observed in the electrospinning of the PLLA membranes. Figure 6 shows wide-angle X-ray diffraction profiles of a cast film of the PLLA raw material and the electrospun PLLA membrane. The cast film showed three crystalline peaks at 16.7, 19.802, and 21.302°, whereas the electrospinning membrane exhibited wider peaks at the same positions, which represented a less perfect crystal structure. These results confirm that crystallization is retarded during electrospinning, and this can be attributed to the rapid solidification of the stretched chains at a high elongation rate during the process of electrospinning. In other words, there is not enough time for the stretched chains to be organized into three-dimensional, ordered crystal structures before they are solidified. With respect to the nanofiber containing carmofur, there is an evident difference between its X-ray curve and that of the nanofiber not containing carmofur. The diffraction peaks are at the same positions but are much narrower, and this suggests that carmofur favors the crystallization of the electrospinning PLLA nanofiber (serving as the nucleus for crystallization).

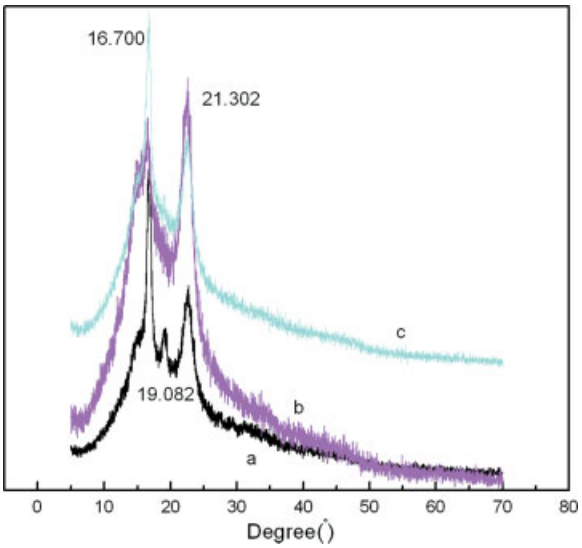

Figure 6 XRD of (a) the solvent-cast film, (b) the electrospinning nanofiber membrane, and (c) the nanofiber with carmofur. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Drug release investigation

Furthermore, the release profiles of various polymer membranes incorporated with carmofur are shown in Figure 7. These samples were PLLA, PLGA, and PLLA/PLA–PEG membranes containing 8% carmofur. All the membranes showed a quick burst of drug release in approximately the first 10 h, and the initial amount of the released drug varied with the different polymer compositions of the membranes. It is worth noting that the PLA–PEG/PLLA membrane showed a larger initial burst and a nearly zero

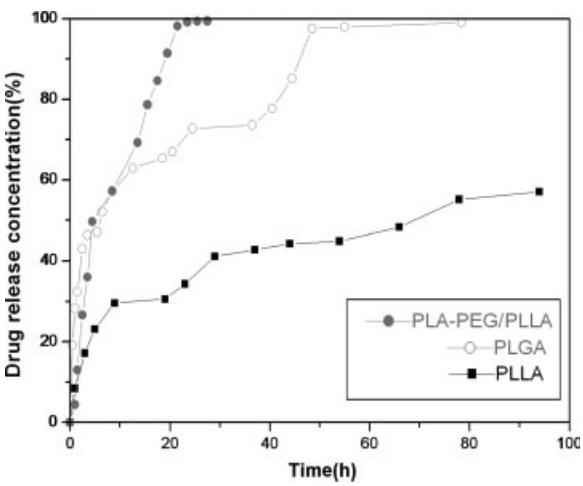

Figure 7 Drug release profiles of different base materials.

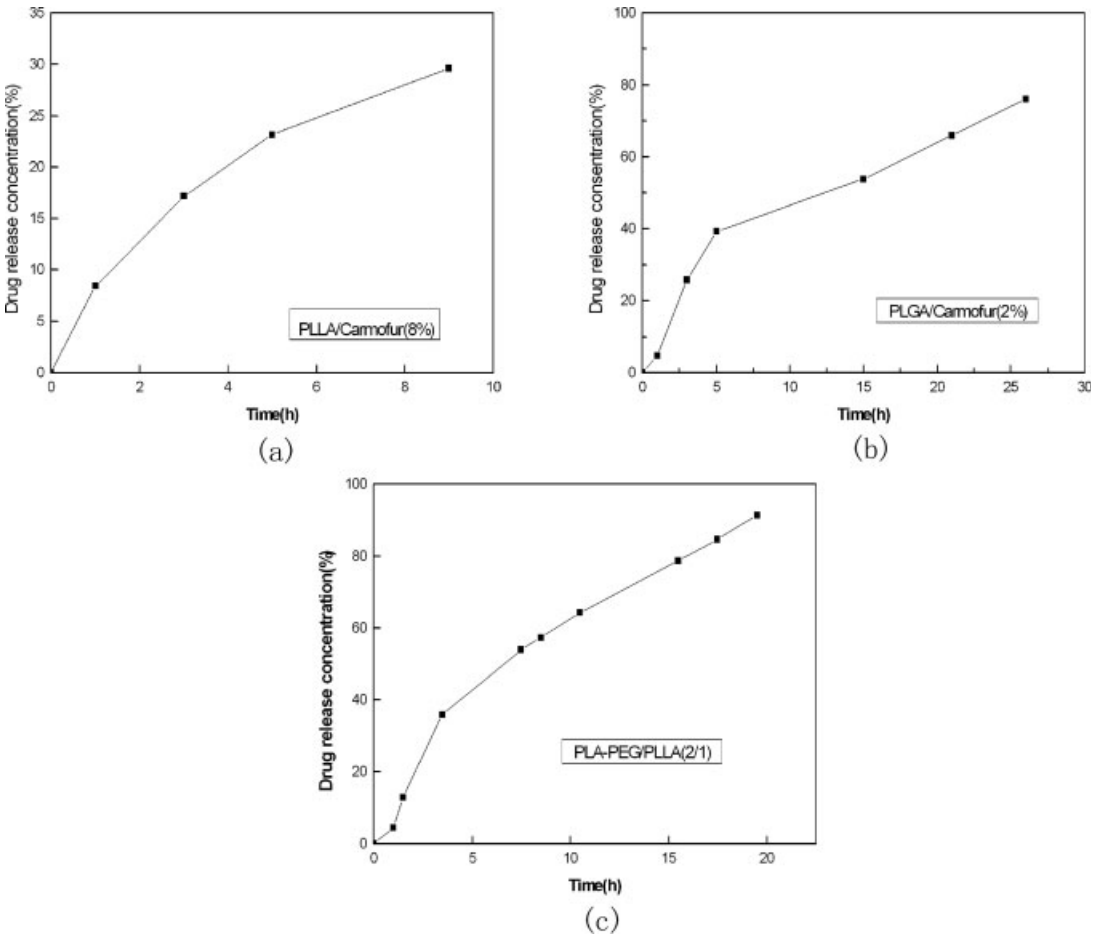

Figure 8 Initial nearly zero kinetic drug release profile of each material scaffold: (a) PLLA, (b) PLGA (85 : 15), and (c) PLA–PEG/PLLA (2/1).

kinetic release profile. That is consistent with a recent study by Kim et al.,[12] who reported a large initial burst of 50% from PLGA/PLA/PLA–PEG (80 : 5 : 15) in the first hour, and about 27% of cefoxitin sodium was released continuously over a week. In this study, PLA–PEG was chosen as the main polymer for electrospun fibers. In such an aqueous release medium, PLA–PEG, a comparatively hydrophilic polymer, showed more reciprocity with the medium. In this study, the initial maximum release occurred in the first 10 h, during which the drug was released at a constant rate. After the initial burst of drug release, there was less release from the PLGA carrier, and it was even flat for the PLLA membrane; afterward, both polymer membranes showed sustained drug release following nearly zero-order kinetics.

The drug release process could be divided into three stages: the initial burst release, a leveling-off stage with a small amount of drug release, and another steady release. During the electrospinning process, there is limited physical interaction between the drug and polymer matrix because the drug carmofur is soluble in both hydrophilic and hydrophobic solvents. Part of the drug is likely localized on the surface of the nanofibers because of the electricity-conducting ability of carmofur. The drug localized on the fiber surface is favorably distributed throughout the whole fiber membrane uniformly, so the drug is first washed away in an aqueous solution in the first 10 h. PLGA membranes, as shown in Figure 8, because of their higher degradability in comparison with PLLA membranes, showed a quick and sustained drug release of about 42% in the next 25 h after burst release, and they released 90% of the drug in 94 h of testing. In comparison with PLGA membranes, the PLLA carrier released nearly 60% of the incorporated drug, and this was mainly due to the slower degradation. It is interesting that the PLLA membrane release profile is a ladderlike curve. There are intervals between the drug releases of each layer of the membrane because of its slow degradation. However, PLA–PEG/PLLA showed a nearly zero kinetic release profile for 20 h.

CONCLUSIONS

The electrospinning technique can be used to fabricate biodegradable membranes for biomedical applications

such as tissue engineering and drug delivery. The morphologies and structures of the membrane are dependent on the solution properties and processing parameters. In this study, the effects of the applied voltage, collector distance, and kind of solvent on nanofiber morphologies were investigated, and the results show that the applied voltage, collector distance, and kind of solvent have significant effects on nanofiber morphologies. Generally, the fiber diameter increases with an increase in the electrospinning voltage and a decrease in the collector distance. A higher concentration and higher charge intensity of the solution favor the formation of uniform nanofibers without beads. The diameters of the nanofibers decrease with the addition of drugs. The electrospinning process results in a decrease in T_g and T_m. Electrospinning retards the crystallization of PLLA significantly. All these changes exert effects on the degradation and mechanical properties of the materials, which are to be further investigated in the future. PLA–PEG/PLLA scaffolds show a faster drug release profile than PLLA and PLGA membranes. Nanofibrous membranes with suitable release profiles can be fabricated from appropriate combinations of PLLA, PLGA, and PLA–PEG.

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