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Morphological Characterization of Electrospun Nano-Fibrous Membranes of Biodegradable Poly(L-lactide) and Poly(lactide-*co*-glycolide)

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Summary: Hydrophobic biodegradable polyesters, poly(L-lactide) (PLLA) and poly(lactide-*co*-glycolide) (PLGA), were electrospun on different types of collectors to induce morphological changes in the nanofibrous membrane. On the metal collector smooth nonwoven membranes were obtained for both PLLA and PLGA, while on the water reservoir the surface of the membranes became rough due to shrinkage and slow charge dissipation. When NaCl was added to water to enhance the conductivity, the roughness of the membrane surface was changed, yet the shrinkage remained relatively unchanged. The crystallization of PLLA electospun material on the metal plate was suppressed because of the rapid solvent evaporation, however, upon annealing above the glass transition temperature for 24 hr the PLLA membrane became crystallized. When electrospun on the water reservoir, the PLLA membrane remained amorphous. Crystalline PLLA was obtained by electrospinning on the methanol reservoir due to the swelling of nanofibers by methanol.

Keywords: electrospinning; liquid reservoir collector; nanofibrous membrane; poly(lactide-*co*-glycolide); poly(L-lactide)

Introduction

Electrospinning is a well-known method of fabricating flexible nonwoven membranes of nanofibers by applying a high electric field to a droplet of polymer solution or melt.[1-3] When the applied electrical force at the surface of the polymer droplet overcomes the surface tension force, a charged jet is ejected. As the solvent evaporates, the charge density increases in the polymer chains, resulting in the bending or whipping of an unstable jet[4,5], which in turn stretches the fiber significantly and eventually forms a nanofiber. The diameter of the fiber ranges between 50 nm to 800 nm. The morphology and the physical properties of electrospun nanofibers can be controlled by changing the processing variables, including the type of the collector. In general, randomly oriented electrospun nanofibers are obtained on metal or glass collectors, but nanofibers can be aligned by

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introducing a specially designed collector. A collector composed of two conductive strips separated by an insulating gap[6] and a collector mounted on a rotational counter electrode[7] have been reported. In addition, a rotating drum was used as a collector to construct large pieces of membranes[8] or carbon nanotube-filled nanofiber yarns.[9]

In this study, we attempted to fabricate a 3-D polymer membrane for biomedical applications by the electrospinning of biodegradable polyesters and investigated the morphological change of electrospun polyesters under different collector systems. Biodegradable polyesters, such as poly(L-lactide) (PLLA) and its copolymer, poly(lactide-*co*-glycolide) (PLGA), were electrospun on the surface of a metal plate and a liquid reservoir of deionized water or methanol, respectively. PLLA and PLGA have been widely studied for biomedical applications including surgical sutures[10], substrates for tissue regeneration[11], and carriers for drug and gene delivery[12,13] because of their biodegradability as well as biocompatibility. PLLA[14] is a semi-crystalline, hydrophobic polymer and possesses a similar tensile strength to polypropylene. On the other hand, PLGA[15], a random copolymer of poly(lactide) and poly(glycolide) (PGA), is amorphous and it is less hydrophobic than PLLA because of the relatively hydrophilic PGA units. The degradation rate of PLGA is also much faster than that of PLLA. However, the cast films of PLLA and PLGA are mechanically brittle and not suitable for cell scaffolding, because they are not porous and cannot facilitate the transport of nutrients and oxygen to the cells. To fabricate a flexible and porous 3-D material suitable for biomedical applications, PLLA or PLGA had to be electrospun using different types of collectors, because the morphology of electrospun fibers may be affected by how the charge dissipates and/or how the fibers stack on the collector surface. For example, on a water surface, hydrophobic electrospun fibers will not uniformly stack due to shrinkage and the slow dissipation of charges. Thus, the final morphology of the electrospun PLLA or PLGA fibers will be completely altered when a metal plate collector is replaced by a water reservoir. Moreover, the crystallization behavior of PLLA may be affected by the type of the collector. The thermal and the mechanical properties of the electrospun nanofibers will also be affected.

Various types of collecting systems were used to electrospin PLLA and PLGA membranes, and the morphological variations were examined by scanning electron microscopy (SEM). Development of the crystalline structure of the crystallizable electrospun PLLA was monitored by a wide angle X-ray diffractometer (WAXD) and differential scanning calorimetry (DSC).

Experimental

Materials. Amorphous poly(lactide-*co*-glycolide) (LA:GA=50:50, PLA50GA50) and crystalline PLLA, both from Alkermes, Inc., were used for electrospinning. The molecular characteristics of PLA50GA50 and PLLA are summarized in Table 1. PLA50GA50 was dissolved in dimethylformamide (DMF) at 37 wt%, and PLLA was dissolved in a mixed solvent of methylene chloride (MC) and DMF (65:35) at 6 wt%. Solvents were purchased from Aldrich and used without further purification.

Table 1. Molecular Characteristics of PLA50GA50 and PLLA.

Polymer	Inherent viscosity (dL/g)	M_w[1] (g/mol)	PDI[2] (M_w/M_n)
PLA50GA50	0.75	75,000	1.69
PLLA	1.6	160,000	1.83

[1] Weight average molecular weight

[2] Polydispersity index

Electrospinning. Both the PLA50GA50 and PLLA solution were electrospun at 20 kV at a steady flow rate of ~10 μl/min using a spinneret with a diameter of 1.2 mm. The spinneret was mounted in the parallel plate geometry as described elsewhere.[16-19] The distance between the spinneret and the collector was 20 cm and the electrospun fibers were collected on a stainless steel plate or a liquid reservoir. The resulting electrospun fibrous membranes were vacuum dried at room temperature for a week to completely eliminate any solvent residue prior to further experiments. Figure 1 depicts the schematic presentation of the electrospinning setup using a liquid reservoir.

Characterizaion. The morphology of nanofibrous membranes was examined by a scanning electron microscope (SEM) (S-4300, Hitachi) equipped with a field-emission gun (10 kV). Samples were gold-coated to minimize the charging effect. The crystalline structure of PLLA was monitored by WAXD (DMAX2500, Rigaku) by scanning the range of 3° – 30° at 5 °/min. DSC (DSC7, Perkin Elmer) was employed to evaluate thermal properties. Five mg of the sample were placed in an aluminum pan and the DSC pan was heated to 200 ℃ at a rate of 20 ℃/min under a nitrogen atmosphere. Dried electrospun nanofibrous membrane was cut into pieces (1 cm x 6 cm). The thickness and weight of each piece were measured for density calculation. In addition, porosity was obtained by using the following equation.[20]

$$\text{Porosity}(\%)=(1-\rho/\rho_0)\times 100$$

where ρ and ρ_0 are the densities of the electrospun membrane and the bulk polymer, respectively.

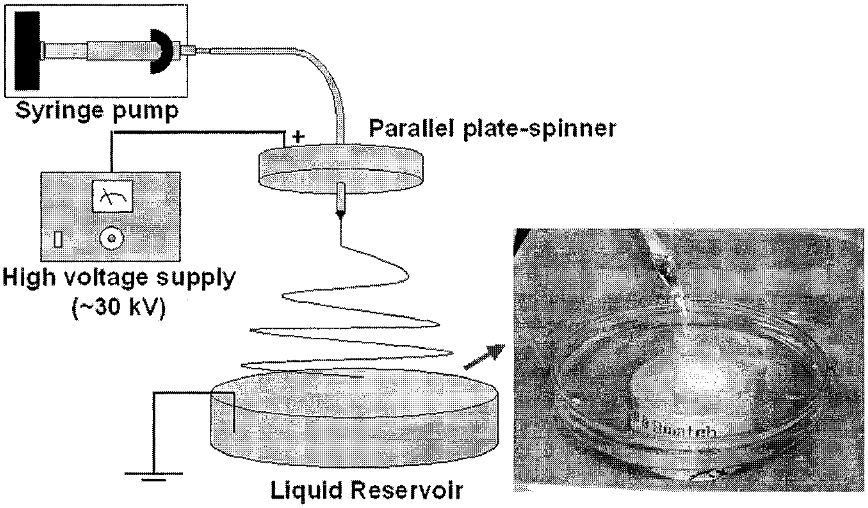

Figure 1. Schematic electrospinning device using a liquid reservoir, shown with a photo.

Results and Discussion

Metal collector. The morphology of electrospun fibers is determined by solution properties and processing parameters. Important solution properties include viscosity (or concentration), conductivity, and the surface tension of the polymer solution. Various processing parameters such as the flow rate, the magnitude of the applied electric field and the distance between the spinneret and the collector should be finely tuned to produce continuous and uniform electrospun fibers. The effect of viscosity or concentration on the structure of the electrospun fiber is shown in Figure 2. Electrospun PLA50GA50 fibers were collected on the metal collector and the morphology of the electrospun fibrous membrane was changed from a bead to a continuous fiber as a function of the polymer

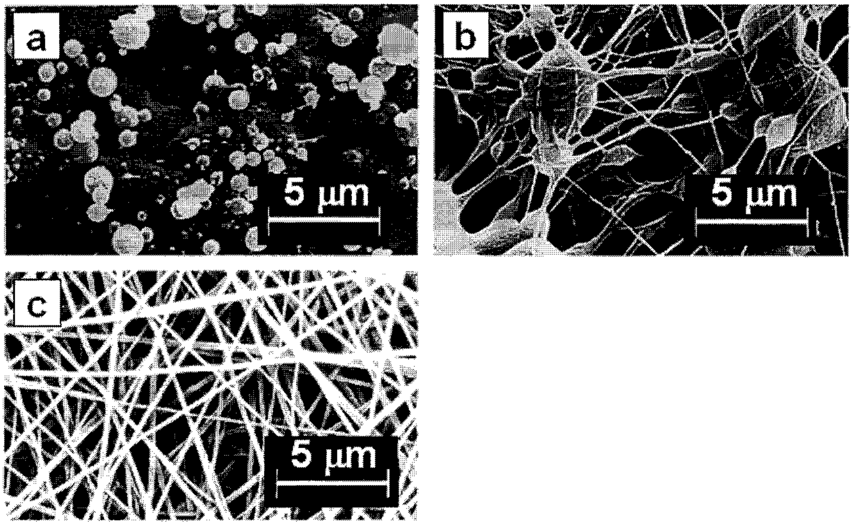

Figure 2. SEM images of the PLA50GA50 membranes electrospun from different polymer concentrations: (a) 20 wt%, (b) 30 wt%, and (c) 35 wt%.

concentration in solution. The average diameter of fibers electrospun from 35 wt% PLA50GA50 solution was 710 nm.

In the case of PLLA, a mixed solvent system of MC and DMF (65:35 by wt%) was utilized to prevent clogging of the spinneret during electrospinning, because the boiling point of MC was low. The surface of both PLA50GA50 and PLLA membranes on the metal collector was smooth because the positive charges in the polymer chains easily dissipated on the conductive collector surface and the stacking of fibers was orderly.

Water reservoir collector. Figure 3 shows the SEM images of the PLA50GA50 nanofiber membranes that were collected on a deionized water reservoir. The average fiber diameter of the electrospun PLA50GA50 was increased to 850±350 nm from 710±200 nm, as the water reservoir replaced the metal plate as collector. It can be assumed that the electric field became unstable because of the fluctuation of the water surface during electrospinning. Meanwhile, hydrophobic PLA50GA50 nanofibers shrank on the water surface and the final morphology of the nanofibrous membrane was drastically different from that collected on the metal collector shown in Figure 2(c). The photos in Figure 3 clearly show the surface ruggedness due to shrinking of nanofibers on the water reservoir.

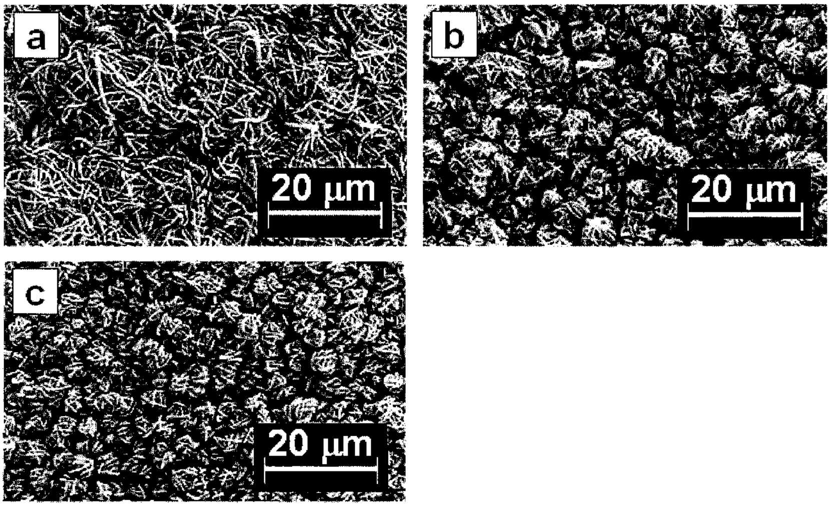

Figure 3. SEM images of electrospun PLA50GA50 membranes: (a) on the water reservoir; (b) and (c) on the water reservoir containing 10% and 20% NaCl, respectively.

As soon as the hydrophobic polymer jet reaches the water surface, it shrinks first and the charge dissipation in the shrunken polymer chains will be slow on the less conductive water surface. Thus, the stacking of electrospun fibers was not regular due to local repulsion, resulting in the surface roughness. If NaCl was added to the water, the morphology of electrospun membranes was changed resulting from the change in conductivity as shown in Figure 3(b) and 3(c). However, the surface of the membrane remained rough because of the shrinkage of nanofibers. The shrinking of the fibers was gradually reduced, as the spinning rate was increased. At high spinning rates, the nanofibers were believed to cover the other fibers even before they shrank on the water surface. The electrospun membranes had a bulk density of 0.1~0.2 g/cm^3 and a porosity of 80~85%, both of which were independent of the collector type.

Methanol reservoir collector. In Figure 4(a) and 4(b), the SEM images of the PLLA nanofibrous membrane collected on the methanol reservoir are shown. The surface of the individual nanofibers was very rough and the fibers were curved, compared to the smooth, rather straight fibers formed on the metal plate (Figure 4(c) and 4(d)). It turned out that methanol wetted the nanofiber surface, so that the PLLA membrane was formed beneath the methanol surface. In the case of PLA50GA50, only large beads were formed on the

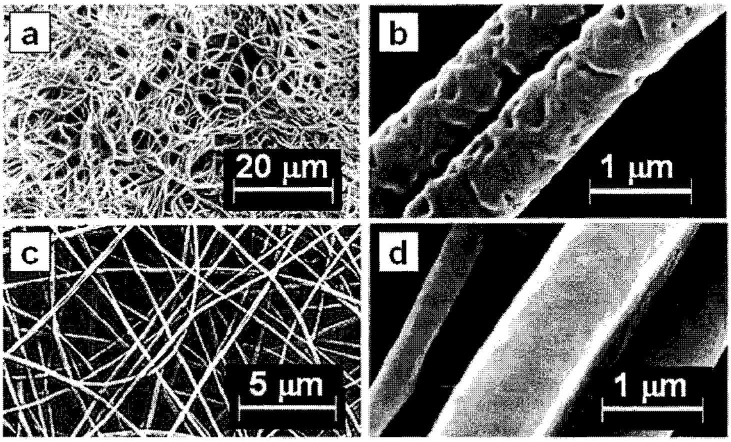

Figure 4. SEM images of electrospun PLLA membranes: (a), (b) on the methanol reservoir; (c), (d) on the metal collector.

methanol reservoir. In order to to correlate the morphology change with the crystallization behavior of PLLA, wide angle x-ray diffraction patterns were taken. Figure 5 shows the diffraction patterns of the PLLA nanofibers electrospun on various types of collectors. Characteristic crystalline peaks of PLLA should be observed at $2\theta=16.5^{\circ}$ and 19°.[21] If the collector was a water reservoir (Figure 5(a)) or metal plate (Figure 5(b)), no crystalline peaks were detected. PLLA could not be crystallized, because the solvent evaporated very rapidly during electrospinning on these collectors. Upon annealing the amorphous PLLA nanofibers electrospun on the metal plate at a temperature higher than its Tg (60℃), crystallization occurred and the crystalline peaks appeared as shown in Figure 5(c). When PLLA was electrospun on the methanol reservoir, its crystalline peaks were observed, although they were somewhat broad (Figure 5(d)), indicating that its crystallization was incomplete. Tsuji *et al.*[22] found that upon swelling PLLA films in different solvents Tg and the crystallinity of PLLA were affected by the solvent. It is believed that the PLLA nanofibers collected on methanol reservoirs became swollen in methanol and then crystallized to some extent. In Figure 6, DSC thermograms of the PLLA membranes are shown to verify the x-ray analyses. The PLLA nanofibers collected on the metal collector show a crystallization peak near 83℃, as seen in Figure 6(a). This means that the electrospinning of PLLA produced amorphous PLLA, in perfect agreement with the WAXD results. Also, the glass transition around 60℃ shows a hysteresis peak due to

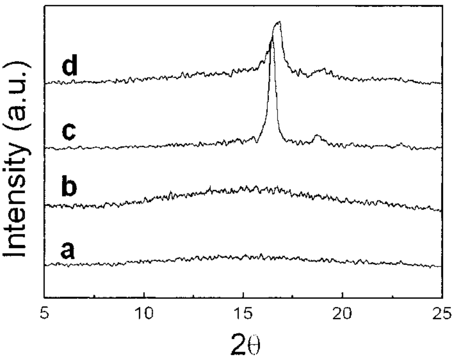

Figure 5. XRD Patterns of electrospun PLLA membranes: (a) on the water reservoir, (b) on the metal collector, (c) on the metal collector, and annealed at 100 ℃ for 24 hr, and (d) on the methanol reservoir.

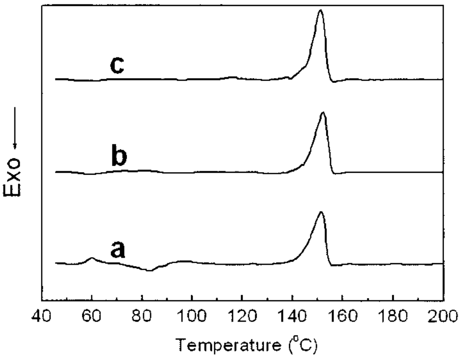

Figure 6. DSC thermograms of electrospun PLLA membranes: (a) on the metal collector; (b) on the metal collector and annealed at 100 ℃ for 24 hr, and (c) on the methanol reservoir.

physical aging[23]. It may have resulted from the quenching effect caused by rapid solvent evaporation. For the nanofibers annealed after electrospinning (Figure 6(b)) or those collected on the methanol reservoir (Figure 6(c)), the melting peak was found to indicate that the crystallization of PLLA had already occurred. Cheng *et al.*[24] reported that the roughness of the material surface might play an important role in cell attachment and cell proliferation. In this study, the rough surface of the biodegradable elecrospun membranes was successfully achieved by employing the liquid resorvoir as a collector, instead of the conventional metal collector. Our ongoing study focuses on cell behavior on the surface of these new electrospun membranes.

Conclusion

Biodegradable polyesters including crystalline PLLA and amorphous PLGA were electrospun on a metal plate and a liquid reservoir, respectively. In the case of the conductive metal collector, the surface of the nanofibrous membrane was very smooth for both PLLA and PLGA. The diameter of the randomly oriented fibers was 0.7-1.0 μm. The crystallization of PLLA was suppressed and no characteristic peak in WAXD or in DSC was observed. The polymer chains are believed to be frozen prior to crystallization because of the rapid evaporation of solvent subsequent to the electrospinning. As the metal collector was replaced by a water reservoir, the hydrophobic nanofibrous membrane shrunk on the water surface and its surface roughness was increased. When the polymer jet reached the water surface, the positive charges in the shrunken polymer chains dissipated slowly. Thus, the stacking of electrospun fibers was irregular due to local repulsion and a wrinkled surface was obtained. Conductivity of water was improved by dissolving NaCl and the surface morphology of the nanofibrous membrane electrospun on the NaCl aqueous solution was altered. When the methanol reservoir was employed as a collector, electrospun fibers soaked up methanol and sank to the bottom of the reservoir. Swelling of PLLA nanofibers in methanol induced the partial crystallization of PLLA, which was confirmed by WAXD and DSC measurements. Nanofibrous membranes of biodegradable polyesters having a rough surface could not only be easily attached to organs without slippage but also make cell culturing possible, as long as the surface roughness can be properly adjusted.

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