Fabrication and Properties of PLLA-Gelatin Nanofibers by Electrospinning

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ABSTRACT: Poly(L-lactide acid)-blend-gelatin (PLLA-gelatin) nanofibers were successfully fabricated by means of electrospinning. The different material components characterizing the properties of electrospun PLLA/G nanofibers were measured and the effect of PLLA weight ratios on such properties as morphologies, physical and chemical structure and mechanical profiles were analyzed. It was found that the fibers diameter increases and the ultimate tension-stress enhances with increased PLLA weight ratio. The analysis of X-ray diffractometry, differential scanning calorimetry, and Fourier-transform infrared spectra demonstrated that the resultant nanofibers from electrospinning of PLLA-gelatin solution are simple blends of these two components. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 542–547, 2010

Key words: electrospinning; PLLA; gelatin; composite; nanofibers blend

INTRODUCTION

In contrast to conventional transplantation methods, the technique of tissue engineering (or regenerate medicine) provided new medical therapy, which used biomaterials with or without living precursor.[1] The ultimate purpose of tissue engineering is to reestablish the destroyed human tissues or organs by provide scaffolds for functional tissue regeneration. In human tissue, extracellular matrix (ECM) is a chemically and physically cross-linked complex network with structural fibers such as collagen fibers and elastin fibers having diameters ranging from several ten to several hundred nanometers.[2] These protein fibers entangle with each other to form a nonwoven mesh which provides tensile strength and elasticity for cells. In addition to providing a physical support, they also create a substrate with for cell adhesion, migration, and regulate cellular proliferation.

Electrospinning technology is a simple and versatile method to prepare ultrathin fibers from polymer solutions or melts. The obtained fibers usually have a diameter from several nanometers to a few micrometers, and mostly in hundreds of nanometers. Therefore, the scaffolds generated from electrospun nanofibers could be capable to mimic the natural ECM.[3] The ultrathin diameters of electrospun nanofibers make them possess large specific surface areas. Additionally, the nonwoven fabrics (mats) from electrospun polymer nanofibers offer a unique capability to control the pore sizes among nanofibers.[4] Unlike nanorods, nanotubes, and nanowires that are produced mostly by synthetic, bottom-up methods, electrospun nanofibers are produced through a top-down nanomanufacturing process, which results in continuous and low-cost nanofibers that are also relatively easy to be aligned, assembled, and processed into applications. Many synthetic and/or natural polymers including, but not limited to, polylactide (PLA),[5] poly(ε-caprolactone),[6] poly (L-lactide-*co*-caprolactone),[7,8] proteins (e.g., collagen),[9] and polysaccharides (e.g., chitosan)[10] have been electrospun into nanofibrous mats as tissue engineering scaffolds.

It has been found that nanofibers made from natural polymers are more positive than those made from synthetic polymers in the interaction between cells and biomimetic ECM, whereas the synthetic polymeric nanofibers have more favorable mechanical characteristics. It is reasonable to expect that an ideal biomimetic ECM should mimic both the mechanical characteristic and the chemical composition of the native ECM. Therefore, many kinds of

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composite nanofibers have been fabricated by electrospinning, such as collagen-blended polycaprolactone[11] and polyaniline-contained gelatin.[12]

PLLA is a biodegradable polymer. It has been investigated as biomaterial for surgery and drug-delivery systems because of its good biodegradability.[13] Gelatin is biologically renewable, biodegradable, nonantigenic, and biocompatible, and has been used in wound[14] healing, drug-delivery carrier,[15] and tissue-engineering applications.[16] In the study of Moon et al., nanofibers nonwoven mats of PLLA-gelatin were fabricated by electrospinning.[17] Furthermore, it has been demonstrated that the addition of gelatin into PLA markedly improved the hydrophilicity of the nanofibrous substrate. The growth of osteoblastic cells was significantly higher on the gelatin-blended PLA than on the pure PLA nanofibers.[18]

In this study, PLLA-blend-gelatin nanofibers were fabricated by electrospinning. The morphologies of fibers varied with the blend ratio of gelatin to PLLA. X-ray diffractometry (XRD), differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) spectra were measured to characterize blended nanofibers.

MATERIALS AND METHODS

Materials

PLLA (M_w = 130,000 g/mol) was purchased from Cargill Co., Ltd. (Minneapolis, MN) Gelatin (porcine skin, Type A) was obtained from Sigma-Aldrich (St. Louis, MO). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was obtained from Darkin Industries., Ltd (Japan). All of the materials were used without further purification.

Electrospinning

PLLA and gelatin were dissolved in HFIP. After the two solutions were prepared, they were mixed together at different blend ratios and stirred for 30 min before electrospinning.

The experimental setup included a high voltage power supply (BGG DC high-voltage generator), purchased from the BMEI Co., Ltd (Beijing, China), and a digitally controlled syringe pump (789100C, Cole-Palmer). During electrospinning, a positive high voltage of 20 kV was applied at the tip of a syringe needle with an inner diameter of 0.9 mm. The electrospun nanofibers were collected on a piece of aluminum foil placed at a distance of 15 cm below the tip of the syringe needle. The mass flow rate was maintained at 1.2 mL/h. The electrospinning was conducted at room temperature.

Characterizations

Morphology of the nanofibers was observed using a scanning electron microscope (SEM, HITACHI S-2360N, Japan) at an accelerated voltage of 15 kV. Before scanning under the SEM, the samples were sputter coated for 90 s with gold using a JEOL JFC-1200 fine coater. The diameter of the fabricated nanofibers was measured based on SEM images using image visualization software ImageJ developed at Upper Austria University of Applied Sciences.

Electrospun nanofibers samples were tested using an X-ray diffraction instrument (D/MAX-2550PC, Rigaku, Japan) under the condition of Cu Kα1, 40 kV and 300 mA. In this work, PLLA, gelatin, and their blend nanofibers were studied using XRD.

FTIR studies were carried out on compressed films containing KBr pellets and products using a FTIR spectrophotometer (Avatar380). All spectra were recorded by absorption mode at 2 cm^{-1} interval and in the wavelength range of 3800–600 cm^{-1}.

The thermal properties of the electrospun fibers were measured by a TA Instruments DSC-822 differential scanning calorimeter (METTLER TOLEDO Company, Switzerland) in a temperature range from 40 to 200°C at a heating rate of 10°C/min.

Mechanical measurements were achieved by applying tensile test loads to specimens prepared from the electrospun fine nanofibrous mats at a concentration of 8 wt % and different blend ratios of gelatin and PLLA. In this study, five specimens were prepared according to the method described by Huang et al.[19] First, a white paper was cut into templates with width × gauge length = 10 mm × 50 mm and double-sided tapes were glued onto the top and bottom areas of one side. Second, the aluminum foil was carefully peeled off and single side tapes were applied onto the gripping areas as end-tabs. The resulting specimens had a planar dimension of width × gauge length = 10 mm × 30 mm. Mechanical properties were tested by a materials testing machine (H5K-S, Hounsfield, UK) at 20°C, relative humidity of 65% and an elongation speed of 10 mm/min.

RESULTS AND DISCUSSIONS

Miscibility in polymer blends is assigned to specific interactions between polymeric components. The most common interactions in the blends are hydrogen bond, ionic and dipole, p-electrons, and charge-transfer complexes.[20] Gelatin can be easily dissolved in water at temperature of above 40°C as an aqueous solution. Such an aqueous solution can be used to produce large diameter gelatin fibers for arresting bleeding purpose by the method of wet spinning. There are very few high polarity organic solvents

available for dissolving this biopolymer. Furthermore, PLLA is easy to dissolve in nonpolar solvent, such as chloroform. Therefore, it is remarkable to find a proper solvent for electrospinning of PLLA-gelatin blends. In this study, HFIP was used to dissolve PLLA and gelatin. HFIP had been used as electrospun solvent for many natural and synthetic polymers.[9,20,21] It has been known that fluorinated alcohols such as trifluoroethanol and HFIP are good solvents for polypeptide biopolymers such as collagen. Matthews et al. used HFIP as a solvent to have successfully electrospun collagen into nanofibers.[22] As gelatin can be considered as denatured collagen, similar fluorinated solvents should be applicable. In this study, we used HFIP as solvent for the purpose of electrospinning of PLLA-gelatin, which affords the ability to directly electrospin gelatin into ultra fine fibers.

The applied voltage was fixed at 20 kV, and the distance between the spinneret tip and the collector was set at 15 cm. The electrospinning of PLLA, gelatin, and PLLA-gelatin blend solution at the concentration of 8% were investigated.

Morphology of PLLA-gelatin nanofibers

Unlike other electrospun systems, the PLLA-gelatin blends nanofibers show changing morphology with different ratios of PLLA and gelatin. In the electrospinning, there are a number of parameters affecting fiber morphology, such as polymer concentration, applied voltage, needle diameter, and the delivery rate of polymer solution.[23] Additionally, the proportions of two polymers also have significant effect on the spinnability of polymer solution and the fiber morphology. Typically representative SEM micrographs photographs of all the nanofibrous mats with different gelatin concentrations are shown in Figure 1. The morphology of electrospun gelatin exhibited a ribbon structure. The higher contents of PLLA generated nanofibers shaped more like a cylinder. When the proportion of PLLA increased to 7 wt %, the structures of as-spun nanofibers were similar to other nanofibers.[24,25] The fiber diameters of PLLA-gelatin (7 wt %, 1 wt %) calculated by an image analysis of SEM images were ranged from 200 nm to 2.1 μm.

HFIP has a low volatility, and may not be able to completely evaporate during electrospinning. The ribbon morphology of electrospun gelatin was caused by evaporation of HFIP after collecting onto aluminum foil. The trace amount of HFIP left-over in the collected nanofibrous mats could enable the movement of gelatin macromolecular chains and further result the ribbon structure of nanofibers. Additionally, the gelatin content also affected the fiber diameter. Gelatin molecules have high dielectric constant, and are likely to be charged during electrospinning. Thus, the electrospinning jet with higher gelatin content is likely to possess higher amount of

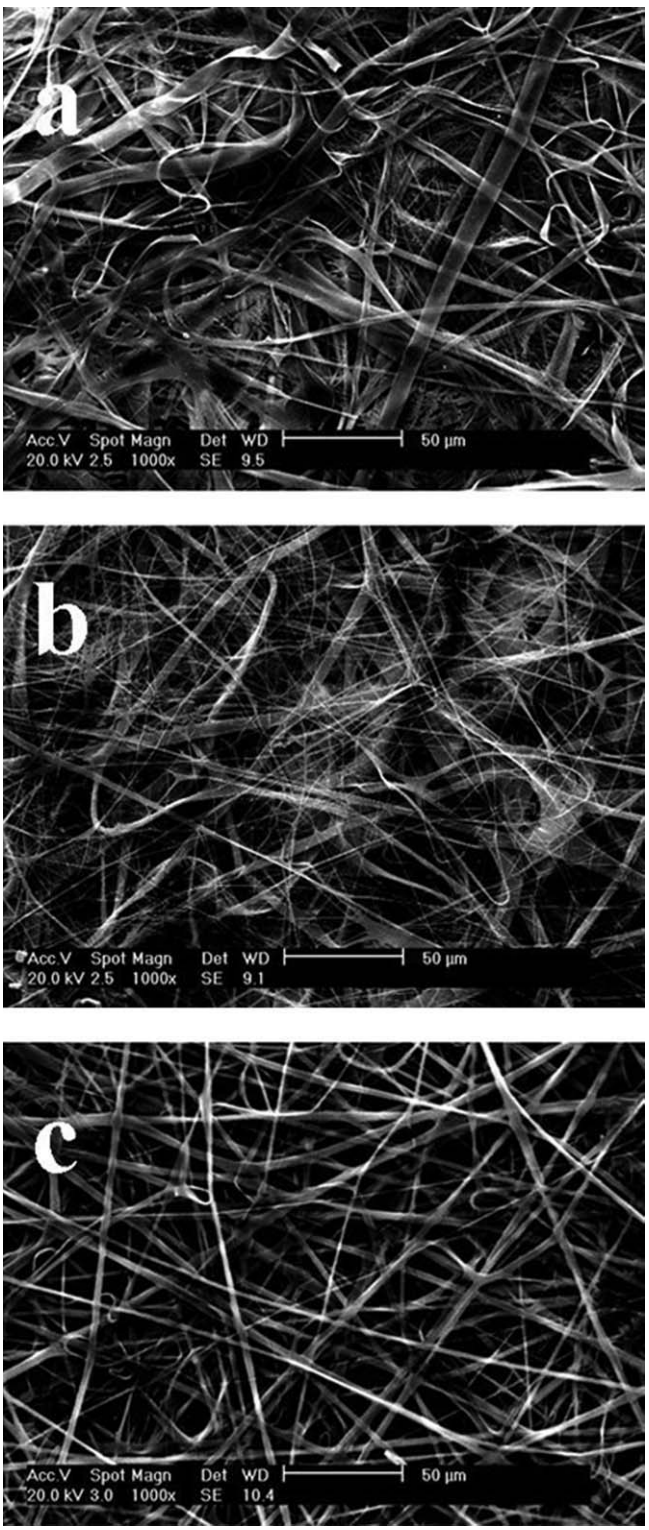

Figure 1 Electrospun nanofibers from different ratios of PLLA-gelatin: (a) 0.08 g/mL gelatin, (b) 0.07 g/m gelatin and 0.01 g/mL PLLA, and (c) 0.01 g/mL gelatin and 0.07 g/mL PLLA.

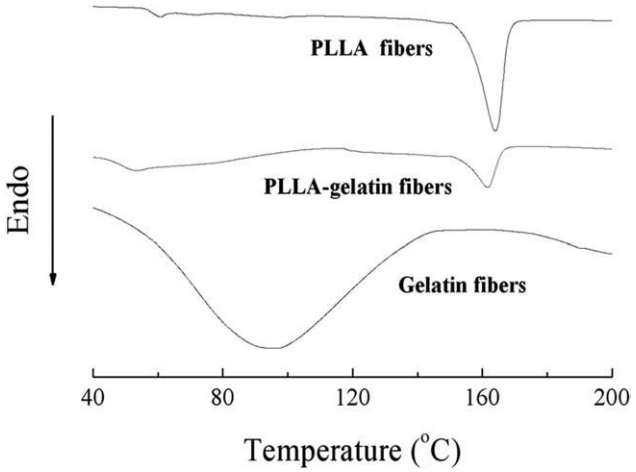

Figure 2 DSC profile of PLLA fibers, PLLA-gelatin fibers, and gelatin fibers.

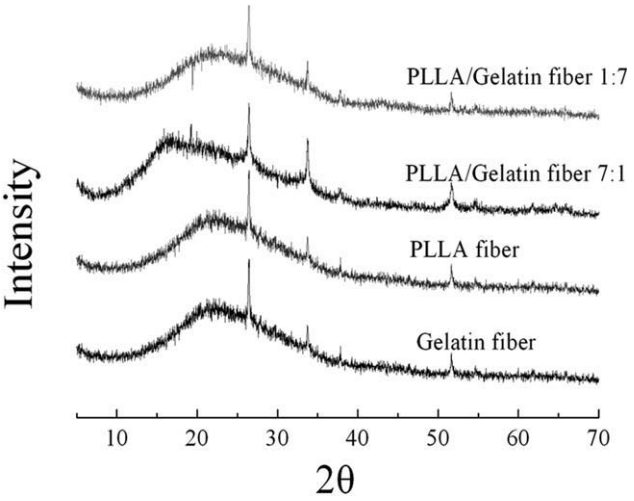

Figure 3 XRD pattern of electrospun gelatin nanofibers, PLLA nanofibers, PLLA-gelatin blend nanofibers with the ratios of 7 : 1 and 1 : 7.

excess charges; and higher amount of excess charges will result in thinner fibers.[8] As shown in Figure 1(a,b), there are a great amount of thin nanofibers company with ribbon structure nanofibers, and the diameter of these nanofibers were smaller than 200 nm.

Thermal behavior of PLLA-gelatin nanofibers

DSC is a widely used thermoanalytical technique. In this work, the technique was used to assess physicochemical properties of PLLA-gelatin nanofibers, such as endothermic or exothermic processes. Figure 2 shows DSC profiles of the electrospun nanofibrous mats of PLLA, PLLA-gelatin and gelatin. The nanofibers were electrospun from 8 wt % solutions at applied voltage of 20 kV. The electrospun nanofibers of PLLA showed a melting point at 167°C. Zong et al.[4] investigated on the DSC analysis of electrospun PLLA nanofibers showing that the polymer chains were noncrystalline, but highly oriented. Furthermore, in Zong's research, the as-received PLLA exhibits a crystallinity of 35.5%, whereas the electrospun PLLA membrane exhibits significantly lower values of crystallinity, shown by the glass transition temperature and the melting temperature. The extent of crystallinity in electrospun PLLA membrane is very low indicating that the majority of the chains are in the noncrystalline state. This was due to the rapid solidification process of stretched chains under high elongation rate during the later stages of electrospinning may hinder the development of crystallinity as the chains do not have time to form crystalline state. PLLA and gelatin are incompatible polymers. The curve relative to PLLA-gelatin blend in Figure 2 clearly shows a broad exotherm (around 80–110° range) due to crystallization of PLLA during heating. Shift of the related melting endotherm to lower values accounts for formation of less perfect crystals in the blend. Moreover, the PLLA glass transition at around 60°C is clearly detectable both in PLLA and PLLA-gelatin curve.

Molecular structure

Figure 3 shows XRD patterns of the electrospun gelatin nanofibers, PLLA nanofibers, PLLA-gelatin blend nanofibers with the ratios of 7 : 1 and 1 : 7. The PLLA and gelatin nanofibers both showed a sharp peak at 2θ of 27.3°, a relatively low intensity peak at 52.7° and a broad peak 2θ around of 22°. Sharp peaks at 27.3 and 52.7° are reasonably attributable to low molecular weight impurities, but not

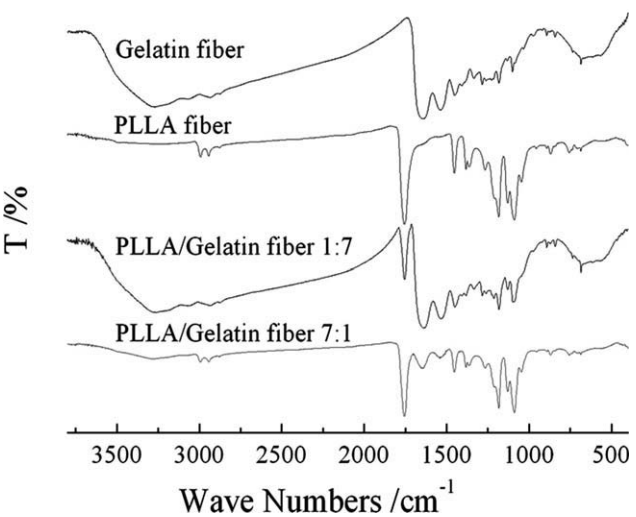

Figure 4 FTIR spectra of gelatin fibers, PLLA fibers, PLLA-gelatin blend nanofibers with the ratios of 7 : 1 and 1 : 7.

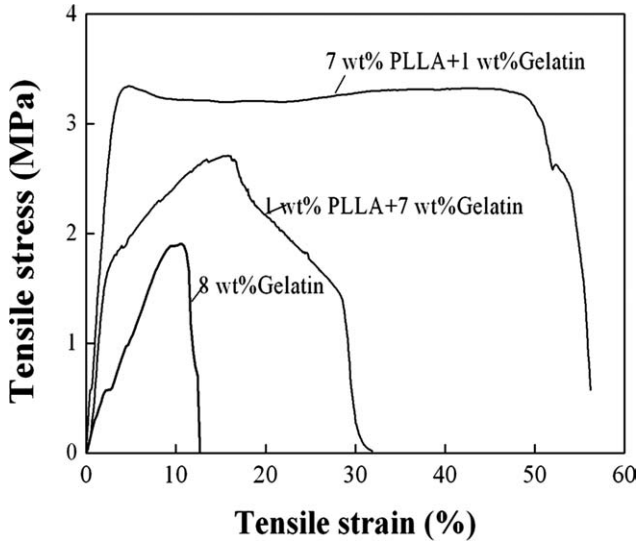

Figure 5 Mechanical curve of gelatin nanofibers, PLLA-gelatin blend nanofibers with the ratios of 7 : 1 and 1 : 7.

to the polymers. For the blend PLLA-gelatin ratio of 7 : 1, the broad peak move from 22° to 2θ around of 16°. The PLLA-gelatin ratio of 1 : 7 presented the same XRD pattern as pure PLLA and/or gelatin nanofibers.

FTIR spectra of electrospun PLLA-gelatin

Figure 4 shows the FTIR profiles electrospun gelatin nanofibers, PLLA nanofibers, PLLA-gelatin blend nanofibers with the ratios of 7 : 1 and 1 : 7, respectively. The FTIR spectra of electrospun gelatin depict characteristic absorption ribbons at 1640 cm^{-1}, 1540 cm^{-1}, and 1250 cm^{-1}, which represent the amide I and II ribbons of gelatin. The PLLA nanofibers depict characteristic absorption ribbons at 1735 cm^{-1} and 1450 cm^{-1} represented the peak of —COOC— and —CH2—. However, there was not new absorption peak in neither of PLLA-gelatin blend nanofibers, which demonstrated that there was no chemical reaction. Therefore, the properties change of PLLA-gelatin nanofibers were mainly caused by van der Waals forces exists between PLLA and gelatin molecular chains.

Mechanical property of electrospun PLLA-gelatin nanofibrous mats

Typical stress–strain curves of pure gelatin nanofibers, PLLA-gelatin blend nanofibers with the ratios of 7 : 1 and 1 : 7 are shown in Figure 5. The details of ultimate stress strength and strain were shown in Table I. It is easy to understand that the pure PLLA nanofibers have the most excellent mechanical properties in the four samples of this work. PLLA-gelatin (7 : 1) nanofibrous mat showed the high module at the initial stage of the force given, and past a yield point with the stress of 3.3 MPa, thereafter, the stress undergoing a plain stage and no further increase, but the strain increased linearly. For PLLA-gelatin (1 : 7) nanofibrous mat, there was also a high module at the initial stage (relative low than PLLA-gelatin 7 : 1), and the yield point was at the stress of 1.7 MPa, thereafter, the stress increased with the increase of strain linearly until the stress was 2.8 MPa. Pure gelatin nanofibrous mat only showed a stiff line and the ultimate stress was 1.9 MPa. As shown in Table I, the average strength of PLLA-gelatin (7 : 1) nanofibrous mat was 3.48 MPa, the average strain was 57.68%, and the module was 42.45; for the PLLA-gelatin (1 : 7) nanofibers, the numbers were 2.45 MPa, 29.74% and 48.76 MPa; for pure gelatin the numbers were 1.86 MPa, 13.4% and 52.4 MPa. The strength of blend PLLA-gelatin nanofibrous mats was attributed to the component of PLLA in fibers, for gelatin contributed much less mechanical performance.

CONCLUSION

Electrospinning is a simple and efficient method to make ultrafine and surface smooth fibers by polymer melts or solutions with diameters ranged from several nanometers up to a few micrometers. In this work, PLLA-gelatin blend was electrospun into nanofibers using HFIP as solvents, we have investigated the blend of PLLA-gelatin nanofibers by electrospinning. Micrographs of SEM demonstrated that nanofibers had smooth surface and the morphology was changed with the ratios of PLLA-gelatin. For mechanical properties, the strength of PLLA-gelatin blend nanofibrous mats were significantly increased

TABLE I
Mechanical Properties of PLLA-Gelatin Nanofibers with Different Blend Ratios

Sample	Breaking strength (MPa)	Breaking strain (%)	Modulus (MPa)
8 wt % Gelatin	1.86 ± 0.42	13.40 ± 5.64	52.4 ± 9.30
7 wt % Gelatin/1 wt % PLLA	2.45 ± 0.57	29.74 ± 8.46	48.76 ± 8.34
1 wt % Gelatin/7 wt % PLLA	3.48 ± 0.78	57.68 ± 11.76	42.45 ± 6.07

compared with pure gelatin nanofibrous mats. Further researches were performed for testing the biocompatiblity of PLLA-gelatin nanofibrous mats.

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