Electrospinning and Crosslinking of Zein Nanofiber Mats

Chen Yao, Xinsong Li, Tangying Song

Biomaterials and Drug Delivery Laboratories, Department of Chemistry and Chemical Engineering, Southeast University, Nanjing 210018, People's Republic of China

Received 21 December 2005; accepted 18 March 2006
DOI 10.1002/app.24619
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Electrospinning processing can be applied to fabricate fibrous polymer mats composed of fibers whose diameters range from several microns down to 100 nm or less. In this article, we describe how electrospinning was used to produce zein nanofiber mats and combined with crosslinking to improve the mechanical properties of the as-spun mats. Aqueous ethanol solutions of zein were electrospun, and nanoparticles, nanofiber mats, or ribbonlike nanofiber mats were obtained. The effects of the electrospinning solvent and zein concentration on the morphology of the as-spun nanofiber mats were investigated by scanning electron microscopy. The results showed that the morphologies of the electrospun products exhibited a zein-dependent concentration. Optimizing conditions for zein produced nanofibers with a diameter of about 500 nm with fewer beads or ribbonlike nanofibers with a diameter of approximately 1–6 μm. Zein nanofiber mats were crosslinked by hexamethylene diisocyanate (HDI). The tensile strength of the crosslinked electrospun zein nanofiber mats was increased significantly. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 380–385, 2007

Key words: biopolymers; fibers; crosslinking

INTRODUCTION

Several methods have been developed to fabricate highly porous biodegradable scaffolds, including fiber bonding, solvent casting, particle leaching, phase separation, emulsion freeze-drying, gas foaming, and 3-D printing.[1] Recently, the electrospinning technique has been recognized as an efficient processing method to manufacture nanoscale fibrous structures for a number of applications. Electrospinning is a process that uses an electric field to control the formation and deposition of polymer nanofibers. Unwoven textiles composed of electrospun fibers with diameters ranging from several micrometers to nanometers have a high surface area–to–volume ratio and morphology similar to natural tissues, making them excellent candidates for use in fabrication of cell-growth scaffolds, vascular grafts, wound dressings, and drug delivery.[2] The large surface area–to–volume ratio allows cellular migration and proliferation in tissue engineering scaffolds. In the past few years a wide variety of both biologically derived and synthetic biodegradable materials have been electrospun to make fibers. In general, materials from natural sources (e.g., collagens from animal tissues) are advantageous because of their inherent properties of biological recognition, including presentation of receptor-binding ligands and susceptibility to cell-triggered proteolytic degradation and remolding. Electrospinning of biopolymers such as collagen,[3,4] elastin,[5] and chitosan has gained a great deal of attention[6] because it can be used to fabricate biomimetic scaffolds for tissue engineering or wound dressing. Unfortunately, fluoro-containing solvents or organic acids are often used to electrospin biopolymers.

Zein, a major protein of corn, has been used in the food industry as a coating material.[7] Zein possesses the additional benefits of being renewable and biodegradable. Zein microspheres have also been investigated for use as carriers to protect drugs from stomach acid. Dong et al.[8] reported that zein films exhibited very good ability to proliferate both human liver cells and murine fibroblast cells. These findings suggested that zein was a promising biomaterial with good biocompatibility. Zein is soluble in aqueous ethanol, an eco-friendly solvent for electrospinning. A primary trial of electrospinning was carried out to produce zein nanofiber mats.[9]But we found that these zein nanofiber mats had very poor mechanical properties.

In this report, we describe how we used ethanol aqueous solutions with a high concentration of zein to electrospin nanofiber mats. To improve flexibility and tensile strength, zein electrospun nanofiber mats were crosslinked by hexamethylene diisocyanate (HDI). This may extend their applications in the biomedical and packaging areas.

Correspondence to: Xinsong Li (lixs@seu.edu.cn).
Contract grant sponsor: National Natural Science Foundation of China ; contract grant number: 50573011.

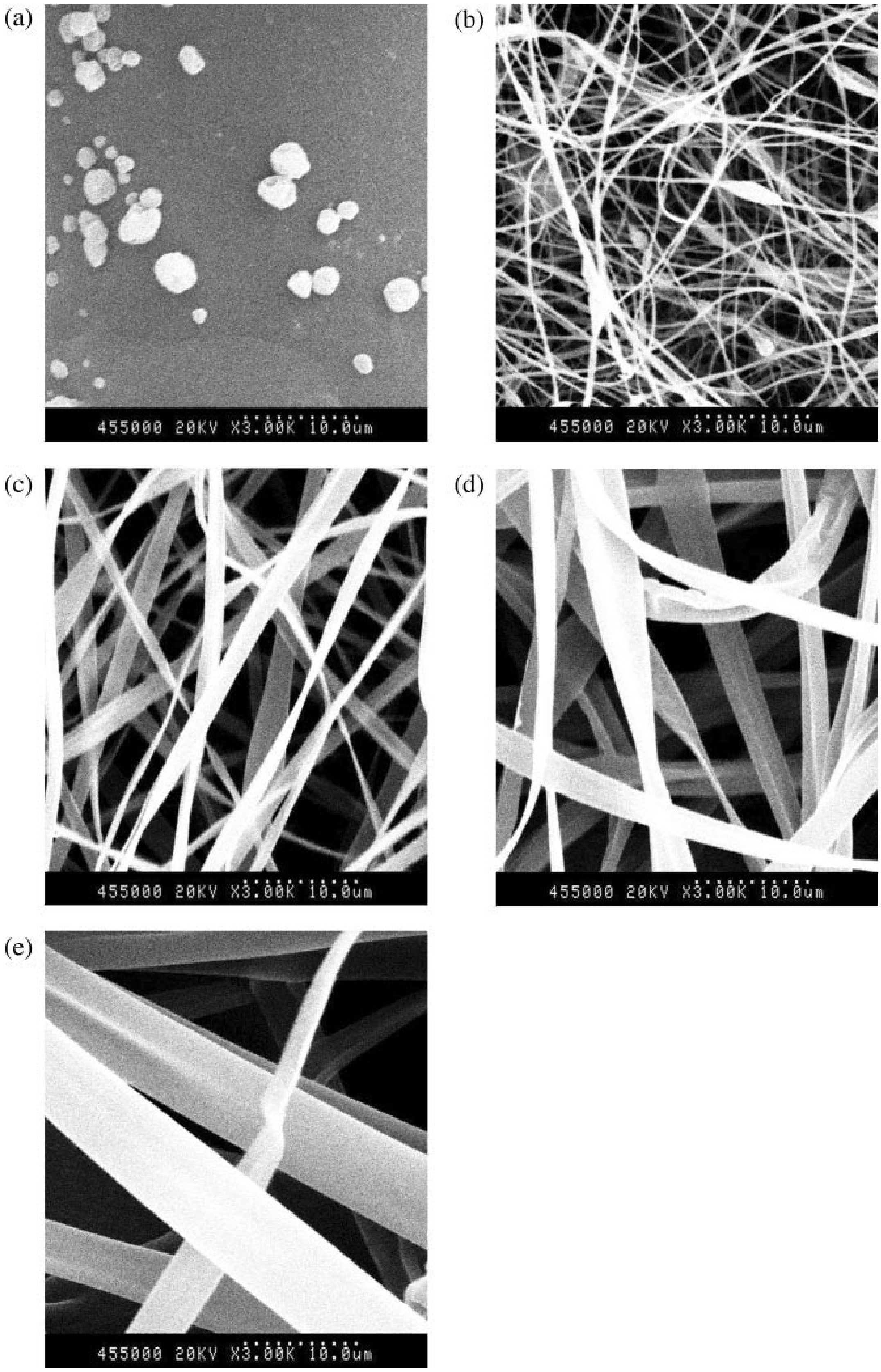

Figure 1 Scanning electron microscope images of zein nanofiber mats electrospun from ethanol aqueous solutions with an ethanol/water ratio of 70 : 30 (v/v) in an electric field of 2 kV/cm and at a zein concentration of (a) 10% (w/v); (b) 20% (w/v); (c) 30% (w/v); (d) 40% (w/v); (e) 50% (w/v) (magnification: 3.00K, scale bar: 10 μm).

EXPERIMENTAL

Materials

Zein (M_w 35,000) was obtained from Scientific Polymer Product Company (Ontario, NY) and used without further purification. Hexamethylene diisocyanate (HDI) was purchased from Aldrich (St. Louis, MO).

Electrospinning of zein

Zein was dissolved in aqueous ethanol with volume ratios of ethanol/water of 70 : 30, 80 : 20, and 90 : 10 (v/v). Aqueous ethanol solutions containing zein in concentrations (g/mL) varying from 10% to 50% (w/v) were prepared. Zein aqueous ethanol solutions were fed into a 20-mL plastic syringe fitted with a needle with a tip diameter of 0.6 mm. A syringe pump was used to feed polymer solution into needle tip, and the feeding rate of the syringe pump was fixed at 6 mL/h. After high voltage in a range from 12 to 20 kV was applied to the needle using a power supply (Tianjing Dongwen High Voltage Electronics Inc.), a positively charged jet of the zein aqueous ethanol solution formed from the Taylor cone and sprayed to a grounded drum. Zein electrospun nanofibers were deposited and collected on the grounded drum, approximately 10 cm from the needle tip. All electrospinning was carried out at room temperature. The as-spun nanofiber mats were dried under vacuum at room temperature.

Crosslinking of zein electrospun nanofiber mats

The as-spun zein nanofiber mats from aqueous ethanol solutions with zein concentrations of 30%, 40%, or 50% (w/v) and an ethanol/water ratio of 70 : 30 (v/v) were crosslinked by immersion in tetrahydrofuran (THF) containing 1 wt % HDI for 10 h, then washed in THF, acetone, and water. The crosslinked nanofiber mats were dried under vacuum.

Characterization of zein nanofiber mats

The morphologies of the as-spun zein nanofiber mats were observed with a scanning electron microscope (Hitachi X-650 SEM) after gold coating. The tensile test was performed using Instron mechanical tester (Model 3345) in room with a constant relative humidity (50%) at 25°C. A crosshead speed of 10 mm/min was used for all the specimens tested. The DSC and TG studies were carried out with a SDT Q600 differential scanning calorimeter (TA Instruments, NY). Data were normally collected between 50°C and 500°C at a scanning nominal rate of 10°C/min.

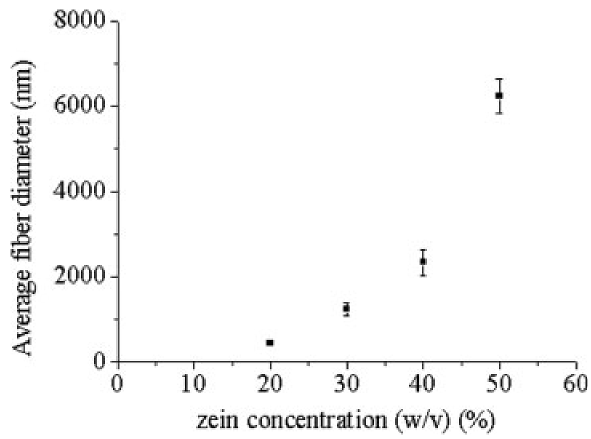

Figure 2 Average diameters of zein fibers electrospun from aqueous ethanol solutions of different zein concentrations with an ethanol/water ratio of 70 : 30 (v/v) in an electric field of 2 kV/cm.

RESULTS AND DISCUSSION

Electrospinning of zein

Aqueous ethanol is a nontoxic, volatile solvent that has been identified as the solvent of choice for the electrospinning of zein. Ethanol aqueous solutions with a zein concentration ranging from 10% to 50 % (w/v) and an ethanol/water of ratio of 70 : 30 (v/v) were electrospun individually in an electric field from 12 to 20 kV/cm to produce nanofiber mats. Figure 1 shows scanning electron microscopy (SEM) images of products electrospun from the zein solutions. Although the concentration of zein was 10% (w/v), microbeads were formed [Fig. 1 (a)]. At higher concentrations, zein nanofibers were obtained. The electrospinning of zein fibers exhibited a concentration dependent on the diameters of the final fibers produced. When the concentration of zein was 20% (w/v), nanofibers with a diameter of about 500 nm were observed, with fewer beads [Fig. 1 (b)]. As the concentration of zein increased from 30% to 50% (w/v), fibers became thicker, and mainly ribbonlike fibers were produced. The diameters of these fibers increased from about 1 to 6 μm.

In this study, solution concentration was found to be the most significant factor controlling the fiber diameter in the electrospinning process. The average diameters of zein fibers electrospun from 20%, 30%, 40%, and 50% (w/v) concentrations are shown in Figure 2. The results indicated that the fiber diameter was dramatically increased with an increase in zein concentration. Increasing the zein concentration in aqueous ethanol increased the solution viscosity, and higher viscosity tended to facilitate the formation of fibers without beads.[12,11] At a higher zein concentration, 50% (w/v), the average fiber diameter was thicker than that at a lower concentration, 30%

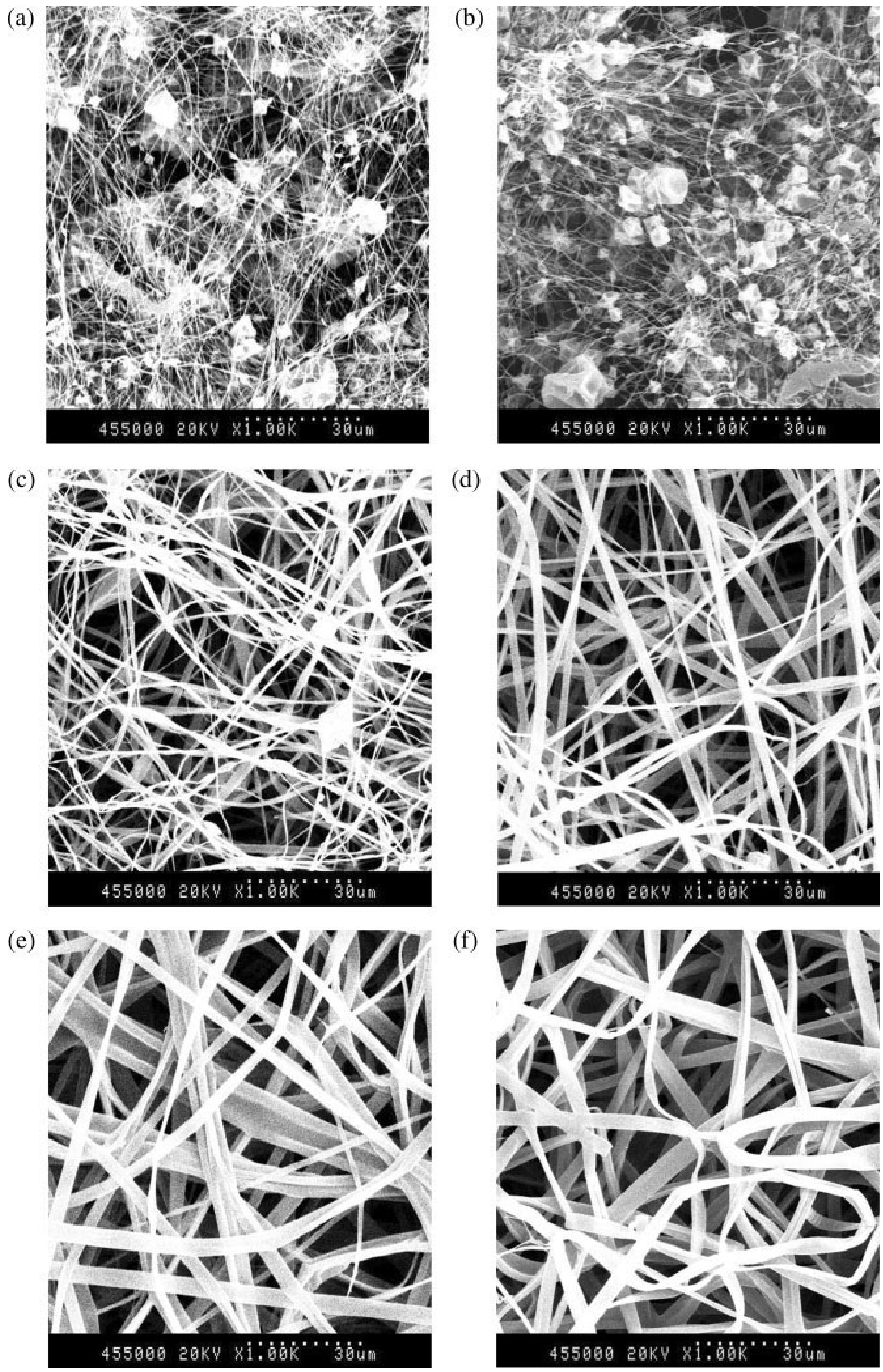

Figure 3 Scanning electron microscope images of zein nanofiber mats electrospun from different concentrations in various solvents: (a) 20% (w/v) in an ethanol/water ratio of 80 : 20 (v/v); (b) 20% (w/v) in an ethanol/water ratio of 90 : 10 (v/v); (c) 30% (w/v) in an ethanol/water ratio of 80 : 20 (v/v); (d) 30% (w/v) in an ethanol/water ratio of 90 : 10 (v/v); (e) 40% (w/v) in an ethanol/water ratio of 80 : 20 (v/v); (f) 40% (w/v) in an ethanol/water ratio of 90 : 10 (v/v) (magnification: 1.00K, scale bar: 30 μm).

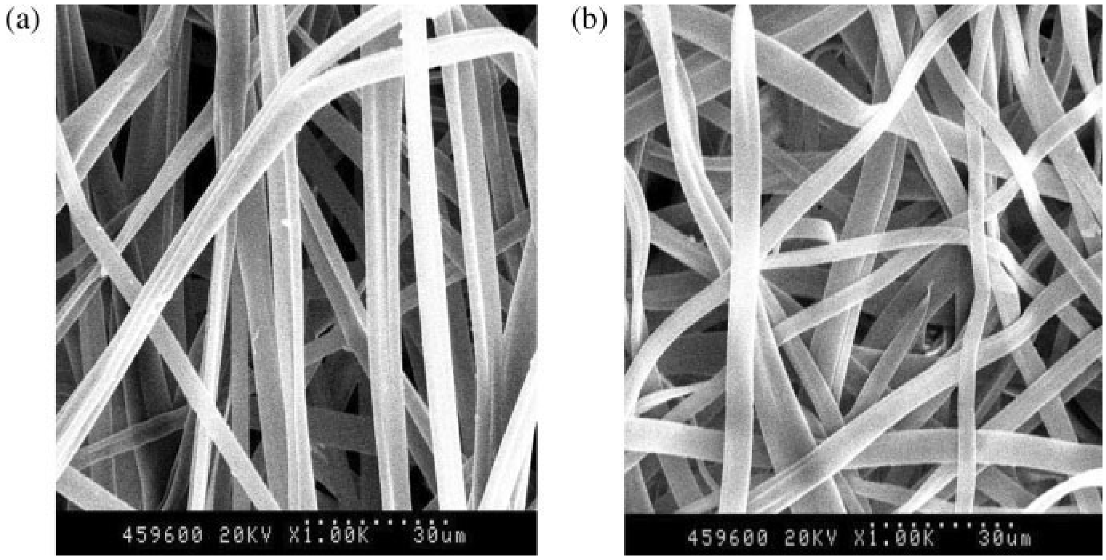

Figure 4 Scanning electron microscope images of nanofiber mats electrospun from 50% (w/v) zein in an ethanol/water ratio of 70 : 30 (v/v) (a) before crosslinking and (b) after crosslinking (magnification: 1.00K, scale bar: 30 μm).

or 40% (w/v), and ribbonlike fibers were observed at various concentration. Reneker et al.[12] first reported the formation of electrospun ribbonlike fibers, and this morphology resulted from collapsing fibers. Miyoshi et al.[9] observed zein ribbonlike fibers, which were attributed to the presence of a thin, mechanically distinct polymer skin on the jets of the polymer solution. Because of the high volatility of ethanol, an outer tube first formed as a skin and then collapsed because the residual solvent evaporated. So the collapsed tubelike skin led to the creation of ribbonlike fibers.[12]

Ethanol aqueous solutions with ethanol/water ratios of 80 : 20 and 90 : 10 (v/v) and zein concentrations of 20%, 30%, and 40% (w/v) were also electrospun, and zein nanofiber mats were obtained. The SEM images of the as-spun nanofiber mats are shown in Figure 3. The morphologies of the nanofiber mats were similar despite the changing composition of the solvent. But zein nanofiber mats electrospun from different solvents showed differences in flexibility. We found that zein fiber mats electrospun from solutions with ethanol/water at a ratio of 70:30 (v/v) were softer and more lustrous. The appearance of the nanofiber mats was estimated to be related to self-assembly of the protein during evaporation of the ethanol.

Crosslinking of zein electrospun nanofiber mats

The nanofiber mats electrospun from 30%, 40%, and 50% (w/v) zein in ethanol/water at a ratio of 70 : 30 (v/v) were crosslinked by HDI, and compact and twisted zein nanofiber mats were obtained. However, the aqueous ethanol solution of 10% (w/v) zein was electrospun to produce microbeads, whereas nanofiber mats from 20% (w/v) zein were fragile and could not be handled because of beads on the fiber surface, which might have reduced the cohesive force between the fibers. Figure 4 shows the typical morphologies of nanofiber mats from 50% (w/v) zein in ethanol/water at a ratio of 70 : 30 (v/v) before and after crosslinking. The crosslinked zein fibers seemed closer and more curved.

TABLE I
Mechanical Properties of Zein Nanofiber Mats Electrospun from an Ethanol Aqueous Solution with an Ethanol/Water Ratio of 70 : 30 (v/v)

Zein concentration of electrospun nanofiber mats (w/v)		Tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)
30%	Uncrosslinked	0.566	37.301	62.094
	Crosslinked	1.095	70.871	14.201
40%	Uncrosslinked	1.696	184.501	7.934
	Crosslinked	4.239	95.154	15.838
50%	Uncrosslinked	0.707	85.714	2.664
	Crosslinked	1.581	16.490	15.606

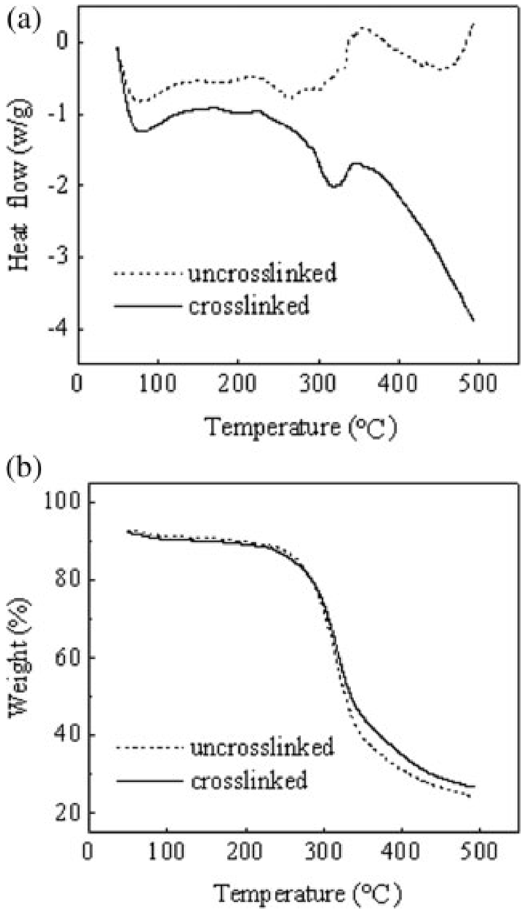

Figure 5 (a) Differential scanning calorimetry (DSC) and (b) thermogravimetry curves obtained for zein electrospun nanofiber mats.

Mechanical properties of electrospun zein nanofiber mats before and after crosslinking are listed in Table I. Zein nanofiber mats electrospun from fibers with a zein concentration of 30% (w/v) showed a small average diameter, were loose and flexible, and exhibited poor mechanical properties. After crosslinking, they showed decreased elongation and increased Young's modulus. Zein nanofiber mats electrospun from a 40% (w/v) concentration showed the highest tensile strength, 4.239 MPa. This may be attributed to a concentration of 40% (w/v), unlike concentrations of 30% or 50% (w/v), producing nanofiber mats with an appropriate fiber diameter and twining structure, resulting in the tightest cohesion. The high tensile strength of zein crosslinked nanofiber mats made from fibers with a 40% (w/v) concentration of zein may extend their applications in the fields of tissue engineering and packaging. The tensile strength and elongation of zein nanofiber mats electrospun from the 40% and 50% (w/v) concentrations were increased with a decrease in Young's modulus after crosslinking. So, it is possible to tailor the mechanical properties of zein nanofiber mats by a combination of electrospinning and crosslinking. The DSC and TG curves of zein nanofiber mats are shown in Figure 5. The results revealed that the crosslinked nanofiber mats had a degradation temperature similar to that before crosslinking. But the crosslinked nanofiber mats underwent endothermic reactions at high temperature.

CONCLUSIONS

Zein nanofibers were produced by the electrospinning of aqueous alcohol solutions. Choosing conditions for zein and electrospinning process produced nanoparticles, nanofiber mats, or ribbonlike nanofiber mats. With an increase in the concentration of zein to make it greater than 30% (w/v), ribbonlike nanofiber mats with diameters of approximately 1–6 μm were obtained. It was concluded that fiber morphology was primarily affected by solution concentration. Zein electrospun nanofiber mats were flexible and lustrous but showed poor mechanical properties. After zein nanofiber mats were crosslinked by hexamethylene diisocyanate (HDI), the tensile strength of the zein electrospun nanofiber mats was increased significantly. So it was possible to improve the mechanical properties of zein nanofiber mats by a combination of electrospinning and crosslinking.

References

1. Cai, Q.; Yang, J.; Bei, J. Z.; Wang, S. G. Biomaterials 2002, 23, 4483.
2. Jin, H. J.; Fridrikh, S. V.; Rutledge, G. C.; Kaplan, D. L. Biomacromolecules 2002, 3, 1233.
3. Matthews, J. A.; Wnek, G. E.; Simpson, D. G.; Bowlin, G. L. Biomacromolecules 2002, 3, 232.
4. Huang, L.; Nagapudi, K.; Apkarian, R. P.; Chaikof, E. L. J Biomater Sci: Polym Ed 2001, 12, 979.
5. Huang, L.; McMillan, R. A.; Apkarian, R. P.; Pourdeyhimi, B.; Conticello, V. P.; Chaikof, E. L. Macromolecules 2000, 33, 2989.
6. Huang, Z. M.; Zhang, Y. Z.; Kotaki, M.; Ramakrishna, S. Compos Sci Technol 2003, 63, 2223.
7. Shukla, R.; Munir, C. Ind Crop Prod 2001, 13, 171.
8. Dong, J.; Sun, Q. S.; Wang, J. Y. Biomaterials 2004, 25, 4691.
9. Miyoshi, T.; Toyohara, K.; Minematsu, H. Polym Int 2005, 54, 1187.
10. Deitzel, J. M.; Kleinmeyer, J.; Harris, D.; Beck Tan, N. C. Polymer 2001, 42, 2612.
11. Fong, H.; Chun, I.; Reneker, D. H. Polymer 1999, 40, 4585.
12. Koombhongse, S.; Liu, W. X.; Reneker, D. H. J Polym Sci, Part B: Polym Phys 2001, 39, 259.