

ELECTROSPINNING OF ZEIN/CHITOSAN COMPOSITE FIBROUS MEMBRANES*

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Abstract Zein/chitosan composite fibrous membranes were fabricated from aqueous ethanol solutions by electrospinning. Poly(vinyl pyrrolidone) (PVP) was introduced to facilitate the electrospinning process of zein/chitosan composites. The as-spun zein/chitosan/PVP composite fibrous membranes were characterized by scanning electron microscopy (SEM) and tensile tests. SEM images indicated that increasing zein and PVP concentrations led to an increase in average diameters of the composite fibers. In order to improve stability in wet stage and mechanical properties, the composite fibrous membranes were crosslinked by hexamethylene diisocyanate (HDI). The crosslinked composite fibrous membranes showed slight morphological change after immersion in water for 24 h. Mechanical tests revealed that tensile strength and elongation at break of the composite fibrous membranes were increased after crosslinking, whereas Young's modulus was decreased.

Keywords: Electrospinning; Zein; Chitosan; Crosslinking; Fibrous membrane.

INTRODUCTION

Electrospinning, a simple and versatile technique, has been popularly recognized as an efficient way to fabricate polymer micro/nanofibers. Nonwoven mats of electrospun nanofibers are well known for their interconnected, three-dimensional porous structures and relatively large surface areas, which make them ideal for the mimic of natural extracellular matrix required for tissue engineering to promote cell adhesion, migration and proliferation. Up to present, nanofibers from various synthetic polymers have been electrospun as well as natural polymers, including proteins, polysaccharides and nucleic acids^[1–3]. Electrospun fibrous membranes of natural polymers, with excellent biocompatible or bioresorbable properties, are extremely acceptable in biomedical applications. However, to convert natural polymer into submicron or nanometer fibers through electrospinning is usually difficult, and fluoro-containing solvents or organic acids are often required. Recently, researchers have investigated electrospinning of polymer blends as candidate materials for biomedical applications because they may facilitate the electrospinning for biopolymers and provide an efficient way to fulfill new requirements for various properties. For example, Duan *et al.*^[4] prepared electrospun PLGA-chitosan/PVA composite membranes for potential use for skin reconstruction. Stitzel *et al.*^[5] fabricated vascular graft scaffolds using electrospun polymer composites of type I collagen, elastin from ligamentum nuchae, and poly(D,L-lactide-co-glycolide). Park *et al.*^[6] produced a matrix composed of chitosan and silk fibroin blend nanofibers *via* electrospinning.

Zein, a prolamin fraction of corn protein, has unique solubility characteristics due to its strongly hydrophobic nature and can be dissolved in aqueous polar organic solvents such as aqueous alcohol solutions.

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The solubility and ready availability of zein make it suitable for numerous biomaterial applications^[7]. Sun *et al.*^[8] revealed that zein can be degraded by both trypsin and collagenase, and the degradation product can enhance cell viability within a certain range of concentrations. Electrospun zein fibrous membranes were investigated as novel scaffolds with potential biomedical applications in the previous literature^[9, 10].

Chitosan is known as one of the most promising biopolymers for tissue engineered scaffolds and wound dressing owing to its excellent biological properties such as biodegradability, biocompatibility, antibacterial and wound-healing activity^[11]. However, conventional chitosan solution formulations display electrical conductivities that are unacceptably large for electrospinning process. Such liquids undergo deep atomization, *i.e.* high charge density, and break up into polydisperse electrosprays^[12]. Many attempts have been made to fabricate chitosan-based fibrous membranes by electrospinning. Chitosan/polyethylene oxide (PEO) composite nanofibers and chitosan/silk fibroin composite nanofibers have been successfully prepared using acid as solvent or compatible polymers as additive^[12, 13].

In the present work, electrospinning of zein/chitosan composite fibrous membrane was explored with the aim to develop novel zein-based biomaterials of broader applications. Poly(vinyl pyrrolidone) (PVP) was introduced to increase the compatibility of zein/chitosan composites and facilitate the electrospinning process. Aqueous alcohol solution was employed as solvent in the electrospinning of zein/chitosan/PVP composites. The electrospun zein/chitosan/PVP fibrous membranes were characterized by scanning electron microscopy (SEM) and tensile tests. In order to improve mechanical properties, zein/chitosan/PVP composite fibrous membranes were crosslinked by hexamethylene diisocyanate (HDI).

EXPERIMENTAL

Materials

Zein ($M_w 3.5 \times 10^4$) was purchased from Scientific Polymer Product Company (Ontario, NY) and used without further purification. Water-soluble chitosan ($M_n 3.0 \times 10^4$) was obtained from Jinan Ocean Biomedical Co., Ltd. Poly(vinyl pyrrolidone) ($M_w 9.0 \times 10^5$) was provided by Hua Kang Biotech Co., Ltd. (Nanjing, China). Hexamethylene diisocyanate (HDI) was purchased from Aldrich (St. Louis, MO).

Preparation of Zein/Chitosan/PVP Aqueous Ethanol Solutions

Stock solutions of zein and PVP were prepared using aqueous ethanol solution with ethanol/water volume ratio of 70/30 as solvent, respectively. Chitosan stock solutions were prepared in water. Zein/chitosan/PVP composite solutions were obtained by mixing stock solutions of zein, chitosan and PVP with vigorous stirring. Composition of the obtained zein/chitosan/PVP solutions is shown in Table 1. Viscosities and conductivities of composite solutions were measured by a digital viscometer (NDJ-9S, ShangPing) and a conductivity meter (DDB-303A, Rex) at room temperature, respectively.

Table 1. Composition of zein/chitosan/PVP solutions

Sample	Concentration (g/L)			Sample	Concentration (g/L)		
	Zein	Chitosan	PVP		Zein	Chitosan	PVP
ZCP1	10			ZCP8		2	
ZCP2	30			ZCP9		10	50
ZCP3	50			ZCP10		20	
ZCP4	100	5	50	ZCP11	50		10
ZCP5	150			ZCP12			30
ZCP6	200			ZCP13		5	70
ZCP7	50	1		ZCP14			90

Electrospinning

The as-prepared zein/chitosan/PVP aqueous ethanol solution was fed by a plastic syringe linked with a spinneret. A syringe pump with a constant feeding rate of 6 mL/h was used to feed polymer solution into the spinneret tip. After a high voltage in a range from 12 kV to 20 kV was applied to the tip using a power supply

(Tianjing Dongwen High Voltage Electronics Inc.), a positive charged jet of zein/chitosan/PVP composite solution was ejected to form nanofibers and deposited onto a grounded drum approximately 10 cm from the tip. The obtained fibrous membranes were dried under vacuum at room temperature.

Crosslinking

The as-spun zein/chitosan/PVP fibrous membranes electrospun from composite solutions with zein concentration of 100, 150 and 200 g/L (ZCP4, ZCP5, ZCP6) were crosslinked by immersing in tetrahydrofuran (THF) solution containing 1 wt% HDI for 10 h. The crosslinked composite fibrous membranes were then thoroughly rinsed with THF and dried under vacuum, followed by immersion in water for 24 h to investigate their morphological changes.

Characterization

Morphologies of electrospun Zein/chitosan/PVP fibrous membranes were observed by using a scanning electron microscope (Mirero AIS2100 SEM) after gold sputter-coating. Average fiber diameter of the composite fibers was evaluated using E-Ruler software program by measuring 100 fibers statistically. Mechanical properties of zein/chitosan/PVP fibrous membranes were determined with a universal testing machine (Instron mechanical tester Model 3345) under crosshead speed of 10 mm/min in a constant relative humidity (50%) room at 25°C. Samples were carefully cut into the standard dumbbell-shaped form. Thickness of the samples was measured with a digital micrometer having a precision of 1 μm .

RESULTS AND DISCUSSION

Electrospinning of Zein/Chitosan/PVP Composite Fibrous Membranes

During electrospinning of zein/chitosan/PVP composites, solution concentration was found to be the most significant factors affecting the structure and diameter of composite nanofibers. Effects of zein, chitosan, PVP concentrations were discussed as follows.

Zein content

Figure 1 shows morphologies of zein/chitosan/PVP fibrous membranes with different zein concentrations and corresponding average diameters of the composite nanofibers. When zein/chitosan/PVP concentration in composite solution was 10/5/50 g/L, beaded fibers with average diameter of *ca.* 260 nm were obtained (Figs. 1a, 1g). As the zein concentration increased to 30 g/L, regular composite fibers without beads were formed and the average fiber diameter was increased to *ca.* 750 nm (Figs. 1b, 1g). It can be seen in Figs. 1(c), 1(d) that thin fibers existed among large ones at zein concentrations of 50 and 100 g/L. Average diameter of the composite fibers increased significantly from *ca.* 2.9 μm to *ca.* 4.8 μm with the increase of zein concentration from 150 g/L to 200 g/L as shown in Figs. 1(e), 1(f), 1(g). Variations in viscosity and conductivity of composite solutions as a function of the zein concentration were investigated. As shown in Fig. 2, viscosity of zein/chitosan/PVP composite solutions increased with increasing zein concentration, while conductivity of composite solutions showed no significant difference. Similar trend of average composite fibers diameter with different zein concentrations is shown in Fig. 1(g). Thus, it is considered that polymer solutions with higher viscosity usually exhibit longer stress relaxation times, which may facilitate the formation of fibers with large diameters during electrospinning^[14].

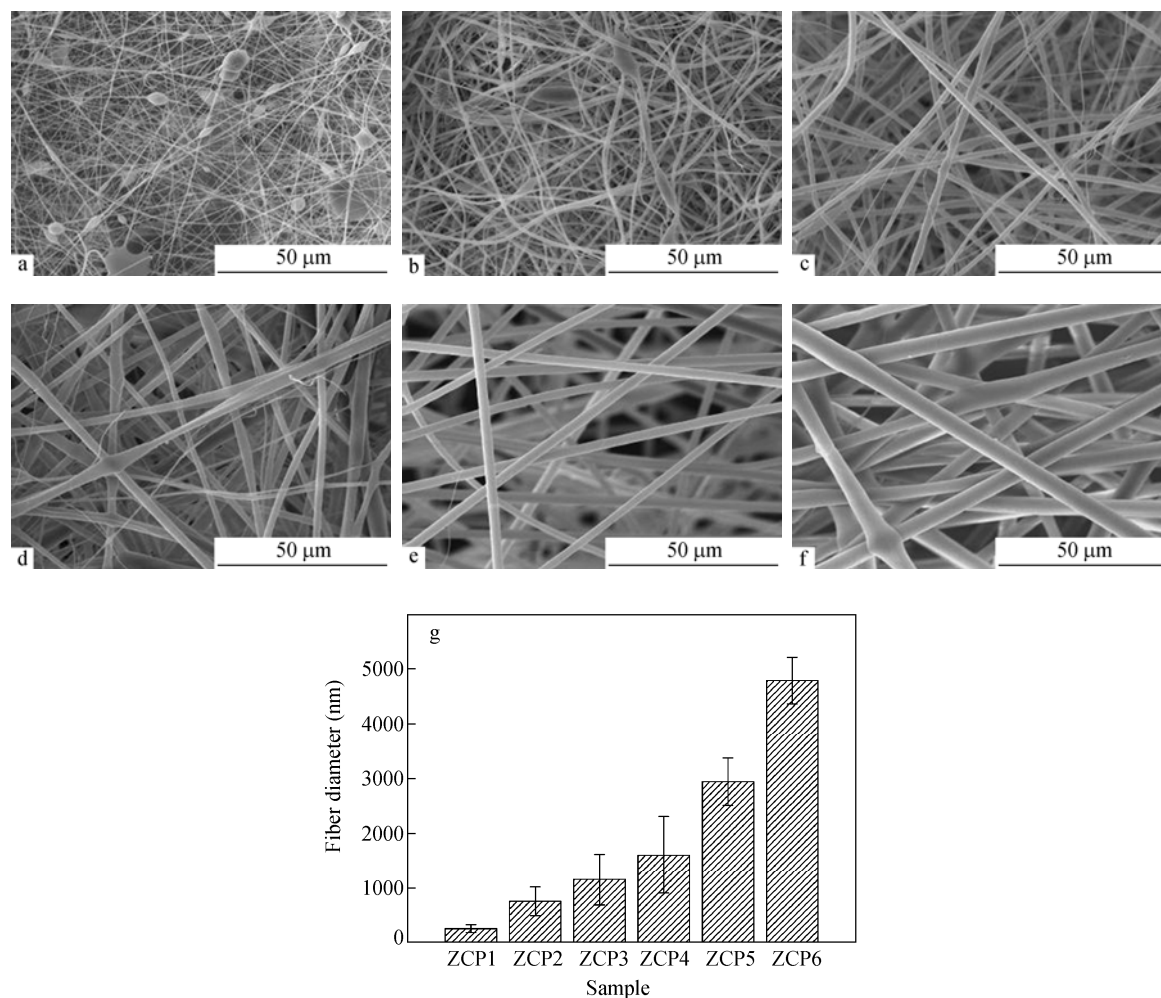


Fig. 1 SEM images of fibrous membranes electrospun from composite solutions with different zein/chitosan/PVP concentrations: (a) 10/5/50 (g/L) (ZCP1), (b) 30/5/50 g/L (ZCP2), (c) 50/5/50 g/L (ZCP3), (d) 100/5/50 g/L (ZCP4), (e) 150/5/50 g/L (ZCP5), (f) 200/5/50 g/L (ZCP6) and (g) average fiber diameters of composite fibrous membranes with different zein concentrations

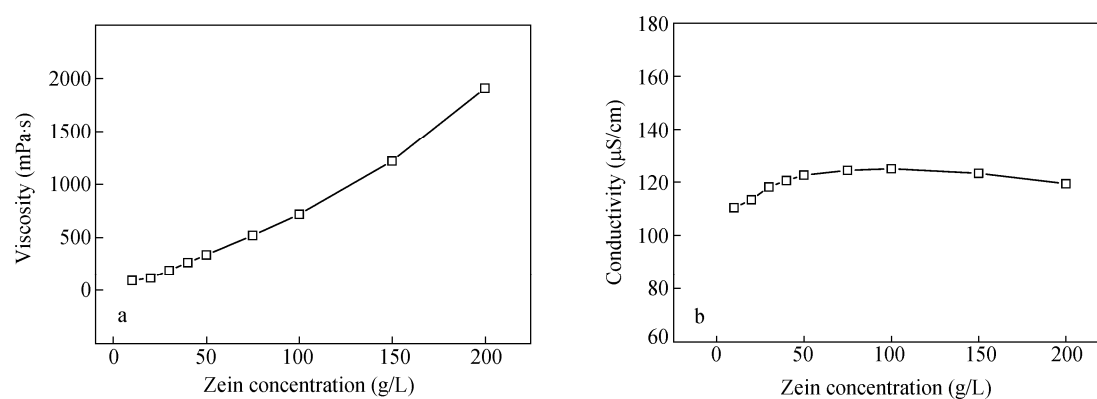


Fig. 2 (a) Viscosity and (b) conductivity of zein/chitosan/PVP solutions with different zein concentration. Concentration of chitosan and PVP was 5 and 50 g/L, respectively.

Chitosan content

Chitosan is used as a good thickener for electrospinning dilute polymer solutions^[13]. In the present work,

zein/chitosan/PVP composite solution with high chitosan content was found too viscous for electrospinning. So, chitosan concentration in the composite solution was varied from 1 g/L to 20 g/L while concentration of zein and PVP was fixed at 50 g/L, respectively.

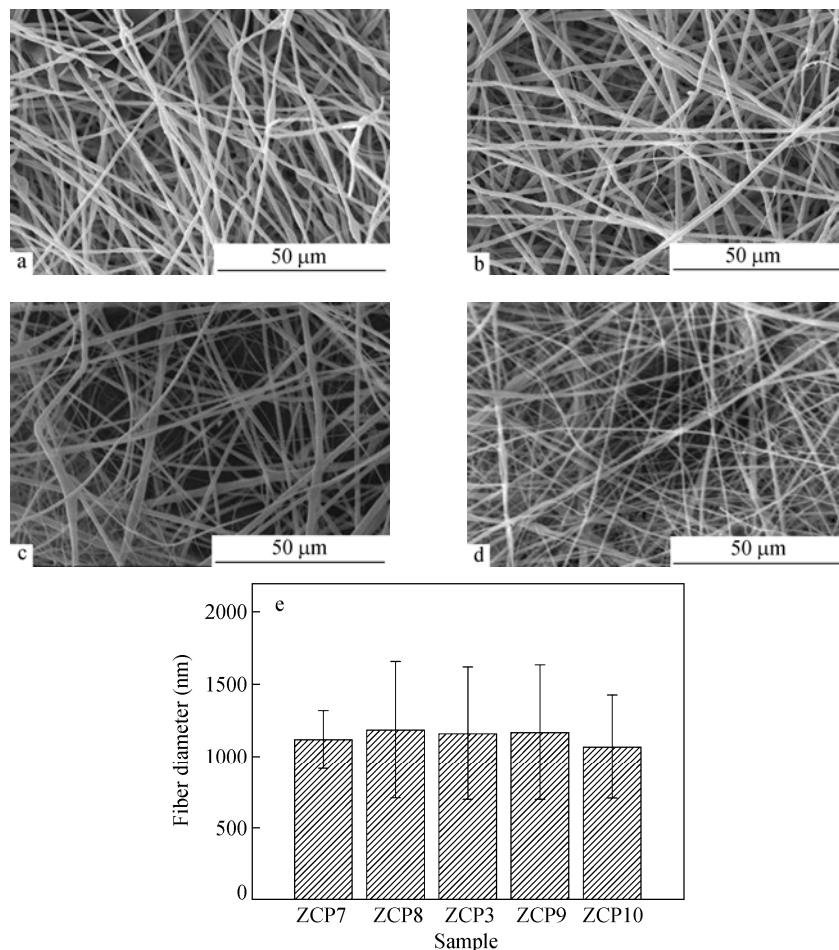


Fig. 3 SEM images of fibrous membranes electrospun from composite solutions with different zein/chitosan/PVP concentrations: (a) 50/1/50 g/L (ZCP7), (b) 50/2/50 g/L (ZCP8), (c) 50/10/50 g/L (ZCP9), (d) 50/20/50 g/L (ZCP10) and (e) average fiber diameters of composite fibrous membranes with different chitosan concentrations

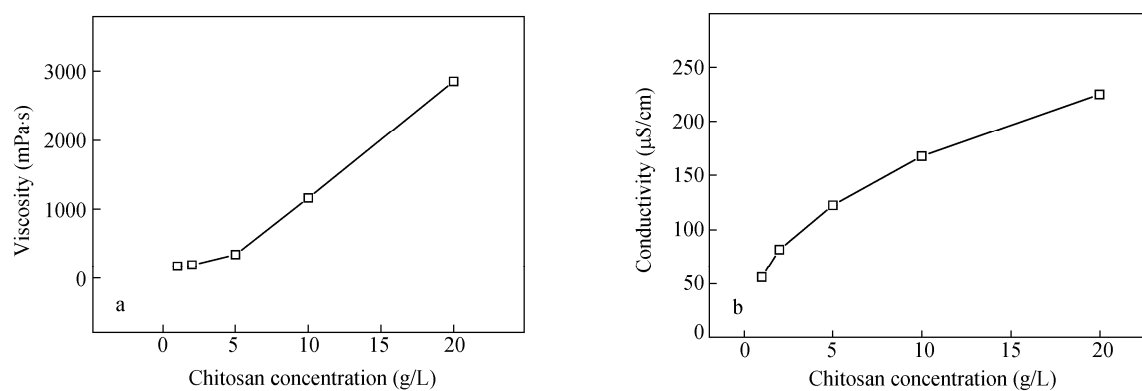


Fig. 4 (a) Viscosity and (b) conductivity of zein/chitosan/PVP solutions with different chitosan concentration. Concentration of zein and PVP were both 50 g/L.

Morphologies of zein/chitosan/PVP composite fibrous membranes with different chitosan concentrations are shown in Fig. 3. Spindle-like nanofibers were obtained at low chitosan concentration of 1 g/L (Fig. 3a). Smooth and continuous fibers were produced as the chitosan concentration exceeded 2 g/L (Figs. 3b, 3c, 3d). But there was no significant difference among average diameters of composite fibers electrospun from solutions with chitosan concentrations of 2, 5, 10 and 20 g/L as indicated in Fig. 3(e). Such inconsistent effect of chitosan concentration on the composite fiber diameters could be ascribed to the simultaneous increase of solution viscosity and conductivity with increasing chitosan concentration as shown in Fig. 4. Higher-viscosity polymer solutions favor the formation of fibers with larger diameters, but higher conductivity could enhance the electric force, which helps to strengthen the whipping instability and leads to the formation of fibers with smaller diameters^[14].

PVP content

In the present work, PVP was introduced to increase the compatibility of zein and chitosan component for preparation of composite solutions. Figure 5 shows SEM images of zein/chitosan/PVP composite fibrous membranes with different PVP concentrations. Beaded fibers were obtained when PVP concentration was

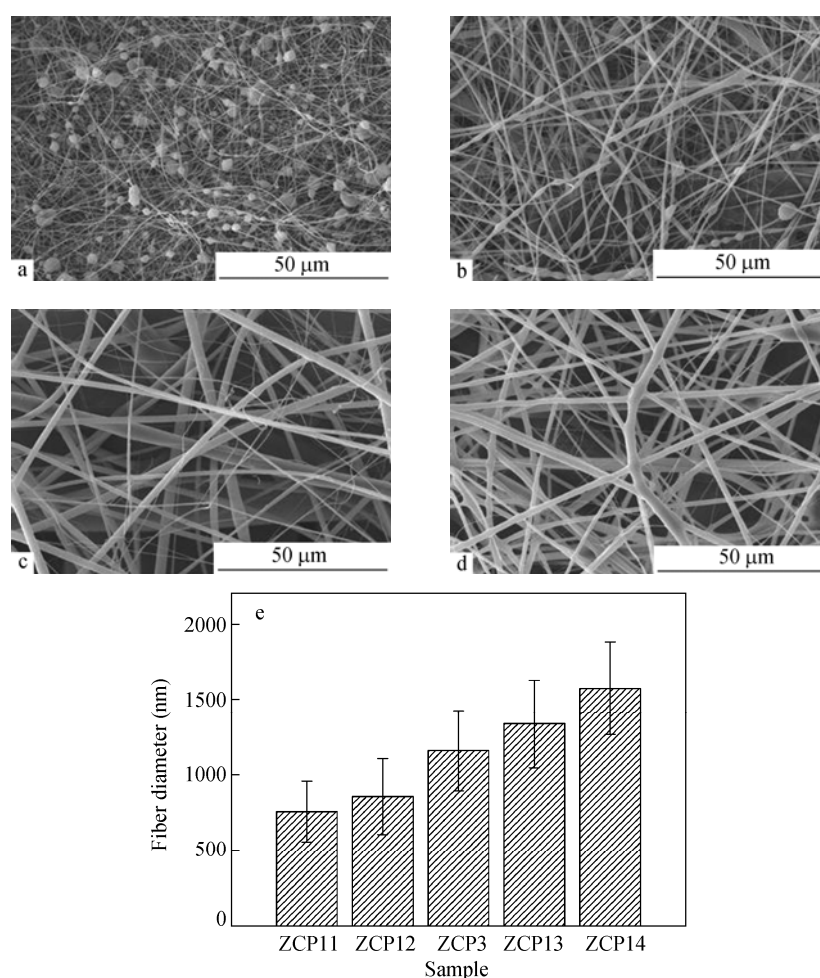


Fig. 5 SEM images of fibrous membranes electrospun from composite solutions with different zein/chitosan/PVP concentrations: (a) 50/5/10 g/L (ZCP11), (b) 50/5/30 g/L (ZCP12), (c) 50/5/70 g/L (ZCP13), (d) 50/5/90 g/L (ZCP14) and (e) average fiber diameters of composite fibrous membranes with different PVP concentrations

10 g/L, and distribution density of beads decreased dramatically as the PVP concentration increased to 30 g/L (Figs. 5a, 5b). Smooth and continuous fibers without beads were produced when the PVP concentration was up to 50 g/L (Fig. 5c). With the increase of PVP concentration from 10 g/L to 90 g/L, average diameters of electrospun composite fibers increased greatly from *ca.* 760 nm to 1.6 μm as indicated in Fig. 5(e). The results were mainly attributed to the variation in viscosity of composite solutions with increasing the PVP concentration (Fig. 6a). It was also notable that increasing PVP concentration led to a decrease in conductivity of the composite solutions (Fig. 6b). This can be explained by the fact that PVP is a non-ionic polymer and prevents ions movement. Since conventional chitosan solutions usually exhibit unacceptable high conductivities, the decreased conductivity by the incorporation of PVP in zein/chitosan composites could facilitate the electrospinning process.

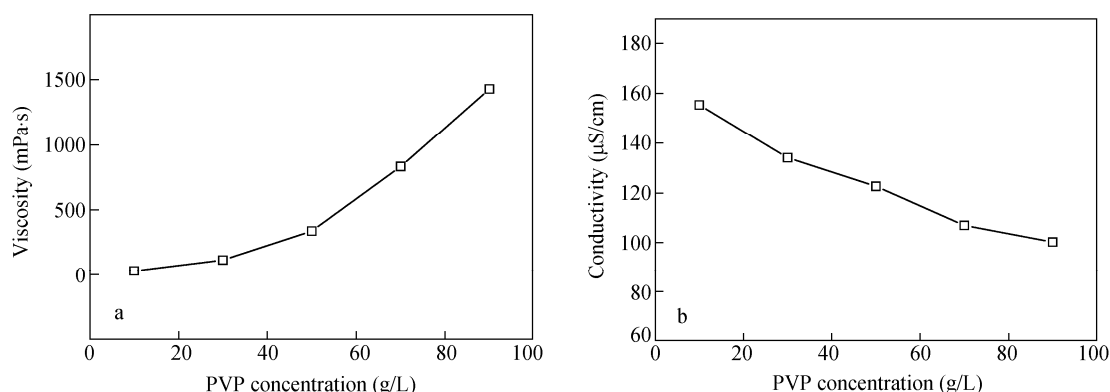


Fig. 6 (a) Viscosity and (b) conductivity of zein/chitosan/PVP solutions with different PVP concentration. Concentration of zein and chitosan was 50 and 5 g/L, respectively.

Crosslinking of Zein/Chitosan/PVP Composite Fibrous Membranes

Pristine zein/chitosan/PVP composite fibrous membranes usually shrink in water and can't maintain their fibrous morphology in wet stage. The fibrous membranes were then crosslinked by hexamethylene diisocyanate (HDI) in order to increase their stability and mechanical properties. The isocyanate groups of the crosslinker reacted with $-\text{OH}$, $-\text{NH}_2$ and $-\text{COOH}$ groups on side-chains of either zein or chitosan. Figure 7 shows SEM images of the crosslinked fibrous membranes electrospun from composite solutions with zein/chitosan/PVP concentrations of 100/5/50, 150/5/50 and 200/5/50 g/L, respectively. Compared with those of pristine composite fibrous membranes shown in Figs. 1(d), 1(e), 1(f), morphologies of the crosslinked membranes are maintained well, and some fibers are fused together after crosslinking and immersion in water for 24 h. Improvement in dimensional stability makes it possible for zein/chitosan/PVP composite fibrous membranes to be used in biomedical areas.

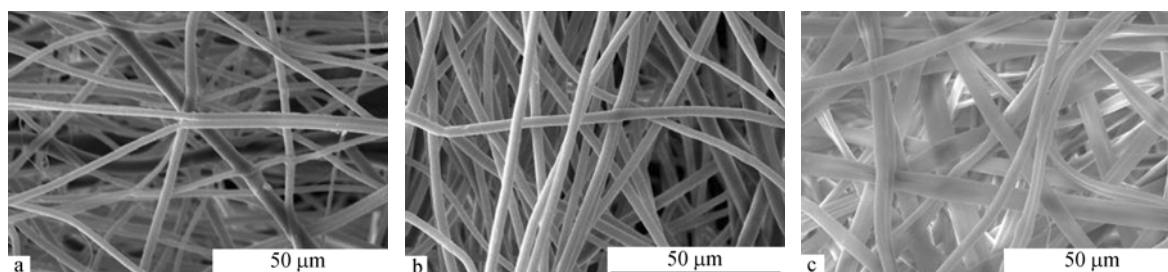


Fig. 7 SEM images of crosslinked fibrous membranes prepared from solutions with different zein/chitosan/PVP concentrations after water immersion for 24 h: (a) 100/5/50 g/L (ZCP4), (b) 150/5/50 g/L (ZCP5), (c) 200/5/50 g/L (ZCP6)

Mechanical Properties

Figure 8 is a typical stress-strain curve of electrospun composite fibrous membranes with zein/chitosan/PVP concentration of 200/5/50 g/L. Tensile strength, elongation at break and Young's modulus of zein/chitosan/PVP composite fibrous membranes before and after crosslinking are obtained from the tensile stress-strain curves and presented in Fig. 9. The tensile strength of composite fibrous membranes was increased gradually from 4.7 MPa to 9.9 MPa with increasing zein concentration from 100 g/L to 200 g/L (Fig. 9a), in agreement with the increase

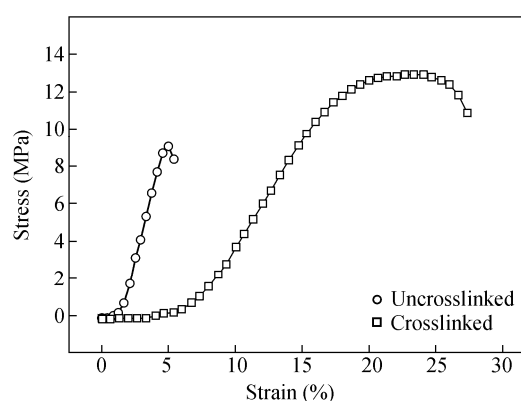


Fig. 8 Tensile stress-strain curve of fibrous membranes electrospun from composite solutions with zein/chitosan/PVP concentration of 200/5/50 g/L (ZCP6)

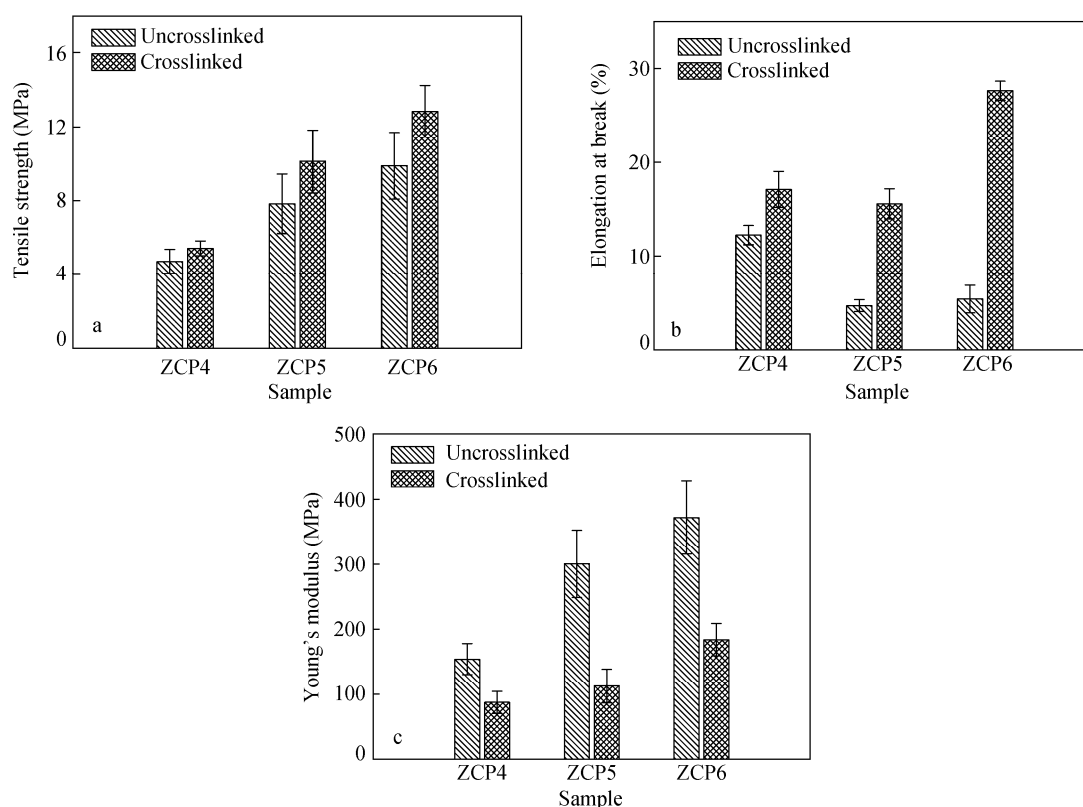


Fig. 9 (a) Tensile strength, (b) elongation at break and (c) Young's modulus of fibrous membranes electrospun from composite solutions with zein/chitosan/PVP concentrations of 100/5/50 g/L (ZCP4), 150/5/50 g/L (ZCP5) and 200/5/50 g/L (ZCP6)

in fiber diameters shown in Fig. 1(g). This is mainly due to the strong “sticky” binding interactions among composite fibers with large diameters^[15]. Besides, tensile strength and elongation at break of the crosslinked composite fibrous membranes were increased, whereas Young’s modulus was decreased. As shown in Fig. 9 (a), composite fibrous membranes electrospun from solution with zein concentration of 200 g/L showed the best tensile strength of 12.8 MPa after crosslinking. The results indicated that crosslinking was an efficient way to fabricate zein/chitosan/PVP composite fibrous membranes with improved mechanical properties.

CONCLUSIONS

Zein/chitosan composite fibrous membranes were electrospun from aqueous ethanol solutions by introducing PVP. Morphologies of the as-spun zein/chitosan/PVP composite fibrous membranes were primarily affected by concentrations of the components. Average diameters of the composite fibers were increased with increasing zein and PVP concentrations, while no significant difference in diameters was shown by changing chitosan concentration. Composite fibrous membranes crosslinked by HDI exhibited slight morphological change after immersion in water for 24 h. Mechanical tests revealed that the tensile strength and elongation at break of the composite fibrous membranes were increased after crosslinking, whereas Young’s modulus was decreased. Crosslinked composite fibrous membranes electrospun from solution with zein/chitosan/PVP concentration of 200/5/50 g/L provided the best tensile strength of 12.8 MPa. The results indicated that crosslinked zein/chitosan/PVP composite fibrous membranes with improved dimensional stability and mechanical properties may be useable as novel scaffolds for biomedical applications.

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