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Electrospinning of methoxy poly(ethylene glycol)-grafted chitosan and poly(ethylene oxide) blend aqueous solution

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ABSTRACT

Methoxy poly(ethylene glycol)-grafted chitosan (PEG-g-CS) was synthesized by mild Michael addition reaction of chitosan and methoxy polyethylene glycol monoacrylate. The chemical structure and degree of substitution (DS) of chitosan derivative were studied by FT-IR and ^{1}H NMR. Blend nanofibers of PEG-g-CS and poly(ethylene oxide) (PEO) were successfully fabricated by electrospinning and characterized by SEM, XRD, DSC and TEM. SEM images showed when the mass ratio of PEG-g-CS/PEO ranged from 4/1 to 1/2, uniform ultrafine fibers could be fabricated. XRD and DSC results confirmed the crystalline microstructure of PEO component. The core–shell structure nanofibers were observed by TEM. Besides, water resistance of glutaraldehyde crosslinked nanofibers and organic solvent resistance of original nanofibers were also evaluated through morphology analysis.

1. Introduction

Electrospinning is a progressive and efficient method to manufacture high performance nanofibers. Both synthetic and natural polymers (hydrophobic and hydrophilic) including poly(lactide-co-glycolide) (PLGA), polycaprolactone(PCL), collagen, cellulose acetate, chitosan, alginate have been successfully electrospun into ultrafine fibers in solvent solution. The nanofibers have been applied in various fields including tissue engineering, drug delivery, wound dressing, nano-sensors, filter media and so forth (Agarwal, Wendorff, & Greiner, 2009; Chen, Chang, & Chen, 2008; Huang, Zhang, Kotaki, & Ramakrishna, 2003; Liao, Chen, Liu, & Leong, 2009; Shalumon et al., 2009).

Chitosan and its derivatives have been successfully electrospun into nanofibers without or with other spinnable polymers (Du & Hsieh, 2007; Geng, Kwon, & Jang, 2005; Jayakumar, Prabaharan, Nair, & Tamura, 2010; Jia et al., 2007; Neamnark, Rujiravanit, & Supaphol, 2006; Ohkawa, Cha, Kim, Nishida, & Yamamoto, 2004). Despite the reported success in fabrication of electrospun chitosan-based fibers, organic solvents or organic acid solvents were employed due to the poor solubility of chitosan, which would limit their the application due to the cost and toxicity of organic solvents. Numerous works have been focused on the modification and functionalization of chitosan to improve its solubility and performances (Mourya & Inamdar, 2008). Grafting poly(ethylene glycol) (PEG) onto chitosan considered as a convenient way to modified chitosan has been extensively investigated. PEG has outstanding physical–chemical and biological properties and therefore has been applied in pharmaceutical and biomedical fields (Li & Kao, 2003). Importantly, PEG is soluble in both water and organic solvents and thus easy for chemical modification. The most typical method for grafting PEG onto chitosan is reductive amination by using PEG-aldehyde. Chitosan was first modified with PEG-aldehyde to yield an imine (Schiff base) and then convert to PEG-g-chitosan via reduction with cyanoborohydride (Bhattarai, Matsen, & Zhang, 2005; Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005; Dal Pozzo et al., 2000; Gorochovceva, Naderi, Dedinaite, & Makuska, 2005; Harris et al., 1984; Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998). However, this scheme involved preparation of PEG-aldehyde which could be readily oxidized by air and using toxic cyanoborohydride. Ouchi, Nishizawa, and Ohya (1998) synthesized PEG-g-chitosan by condensation (coupling) reaction between 6-O-triphenylmethyl chitosan with MeO-PEG acid and then deprotection of triphenylmethyl groups. Gorochovceva and Makuska (2004) prepared O-PEGylated chitosan by etherification between N-phthaloyl chitosan and PEG monomethyl ether iodide using Ag_2O as catalyst. Hu, Jiang, Xu, Wang, and Zhu (2005) synthesized N-PEGylated chitosan by N-substitution of triphenylmethyl chitosan with MeO-PEG iodide in organic medium and subsequent removal of triphenylmethyl groups. Lebouc, Dez, Desbrieres, Picton, and Madec (2005) reported grafting of PEG functionalized by ester groups (MeO-PEG-ester) onto chitosan under different

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reaction conditions. PEG-g-chitosan derivatives were also prepared by using methoxy PEG sulfonate (Amiji, 1997) and methoxy PEG-nitrophenol carbonate (Jiang et al., 2006; Saito, Wu, Harris, & Hoffman, 1997)

Previous researches on PEG-g-chitosan were focused on synthesis method and improvement of solubility. Their application studies were concentrated on hydrogel material and drug delivery system (Bhattarai, Ramay, et al., 2005; Prego et al., 2006; Wu, Wei, Wang, Su, & Ma, 2007). Electrospinning of chitosan and its derivatives has been of particular interest with respect to their potential biomedical application due to their excellent physicochemical and biological properties. Du and Hsieh (2007) synthesized PEGylation chitosan and studied electrospinning of various PEGylation chitosan in organic solutions. Electrospinnability of PEG-g-chitosan blended with other polymer from aqueous solution has not been reported. The objective of this study was to synthesize water-soluble PEG-g-chitosan (PEG-g-CS) through simple and mild Michael addition reaction of chitosan and methoxy polyethylene glycol monoacrylate (MeO-PEG-acrylate) for preparing nanofibers by electrospinning from aqueous solution.

2. Experimental

2.1. Materials

Chitosan (200 kDa, about 88% deacetylated) was purchased from Zhejiang Golden-Shell Biochemical Co. Ltd. (Yuhuan, Zhejiang, China). Methoxy polyethylene glycol (350) monoacrylate (MeO-PEG-acrylate) was kindly supplied by Sartomer Company Inc. (Guangzhou, China). Poly(ethylene oxide) (PEO) with molecular weight of 900,000 g/mol was purchased from Aldrich Co., Ltd. Other reagents and solvents were purchased from Beijing Chemical Reagent Company and used without further purification.

2.2. Synthesis of PEG-g-CS

PEG modified chitosan derivative was synthesized by Michael addition reaction according to the literature (Sashiwa, Kawasaki, et al., 2003). Chitosan (3.0 g) was dissolved in 150 mL acetic acid solution containing 1.0 g acetic acid. After the chitosan was totally dissolved, 10.5 g MeO-PEG-acrylate was added dropwise. The mixture was stirred for 48 h at 50 °C. Subsequently, saturated $NaHCO_3$ solution was added to adjust the pH of reaction solution to 8–9. The solution was then poured into acetone to obtain light yellow product. Finally, the product was dialyzed for 3 days and then lyophilized.

2.3. Characterization of PEG-g-CS

FT-IR spectra of chitosan, MeO-PEG-acrylate and PEG-g-CS derivative were recorded by a Nicolet 5700 FTIR spectrometer (Nicolet Instrument, Thermo Company, Madison, USA). All samples were prepared as KBr pellets and scanned over the wavenumber range of 4000–650 cm^{-1} at a resolution of 4.0 cm^{-1}.

^1H NMR spectra were measured by using a Bruker AV600 MHz (Bruker, Rheinstetten, Germany). Chitosan, methoxy polyethylene glycol (350) monoacrylate and PEG-g-CS derivative were dissolved in D_3CCOOD/D_2O or D_2O according to their solubility.

2.4. Electrospinning procedures

Transparent solutions of 8 w/v% PEG-g-CS and 4 w/v% PEO were prepared separately by dissolving PEG-g-CS or PEO in distilled water with stirring. Then the electrospinning solution was prepared by mixing the two solutions with different mass ratios of PEG-g-CS to PEO. The resultant mixtures were stirred for 2 h and then centrifuged to remove air bubbles before electrospinning. The detailed electrospinning procedure was similar to our previous work (Zhang et al., 2009). Briefly, a DC voltage of 20 kV was applied between the syringe tip and aluminum collectors. The syringe was capped with a needle having an inner diameter of 0.47 mm. The typical distance between the syringe tip and the grounded collector was about 20 cm.

The electrospun nanofibers were collected as a fibrous mat. The fibrous mat was further crosslinked by using 50% vapor phase of glutaraldehyde in a chamber for 48 h and then immerged in distilled water for 1 or 48 h to remove the unreacted glutaraldehyde. Finally, the crosslinked membranes were dried overnight under vacuum. The uncrosslinked fibrous mats were immerged in ethanol for 2 or 48 h to characterize solvent resistance.

2.5. Characterization of nanofibers

The morphologies of fibrous mats were observed by using a scanning electron microscope (Hitachi S-4700, Hitachi Company, Japan). The specimens were fixed on stubs and sputter-coated with gold before observation. The average fiber diameter and diameter distribution were determined by randomly measuring the diameters of the nanofibers at 100 different points from SEM images.

Wide-angle X-ray diffraction (WAXD, D/Max 2500 VB2+/PC, Rigaku Company, Tokyo, Japan) was utilized to reveal the crystal structure of the electrospun nanofibers. The XRD patterns of samples were recorded with area detector operating at a voltage of 40 kV and a current of 50 mA using Cu Kα radiation (λ = 0.154 nm). The scanning rate was 1°/min and the scanning scope of 2θ from 5° to 50°.

The thermal analysis of electrospun fibrous mats was made with a differential scanning calorimeter (TA 2920 Modulated DSC, TA instruments, USA) with a heating rate of 10 °C/min ranged from 0 to 160 °C.

The core–shell structure of the nanofibers was characterized by transmission electron microscopy (TEM, S800 Hitachi, Tecnai G^2 20 S-TWIN FEI, JEM-100CX JEOL).

3. Results and discussion

3.1. Synthesis of PEG-g-chitosan

The schemes for PEG grafting onto chitosan including reductive amination, condensation reaction have some disadvantages including organic medium, complex or toxic catalyst, protection and deprotection of groups, which limits chitosan derivatives utilization in biomedical filed. Since the grafted polymers are designed for biomedical application, it is desirable to develop simple and convenient schemes. Michael addition reaction as a facile method was developed to functionalize chitin or chitosan. A series of chitin and chitosan derivatives were synthesized through Michael addition between amino groups in chitosan and various acryl reagents (Aoi et al., 2000; Ma et al., 2008; Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003).

In present research, PEG-g-CS derivative was synthesized through Michael addition between amino groups in chitosan and methoxy polyethylene glycol monoacrylate. Amino compounds are used as a nucleophile of a Michael-type addition of α,β-unsaturated carbonyl compounds. Ther reaction of chitosan with MeO-PEG-acrylate was carried out at 50 °C.

3.2. Characterization of PEG-g-CS

The chemical structure of product was determined by FT-IR and ^1H NMR spectroscopies. A comparative IR spectrum study of

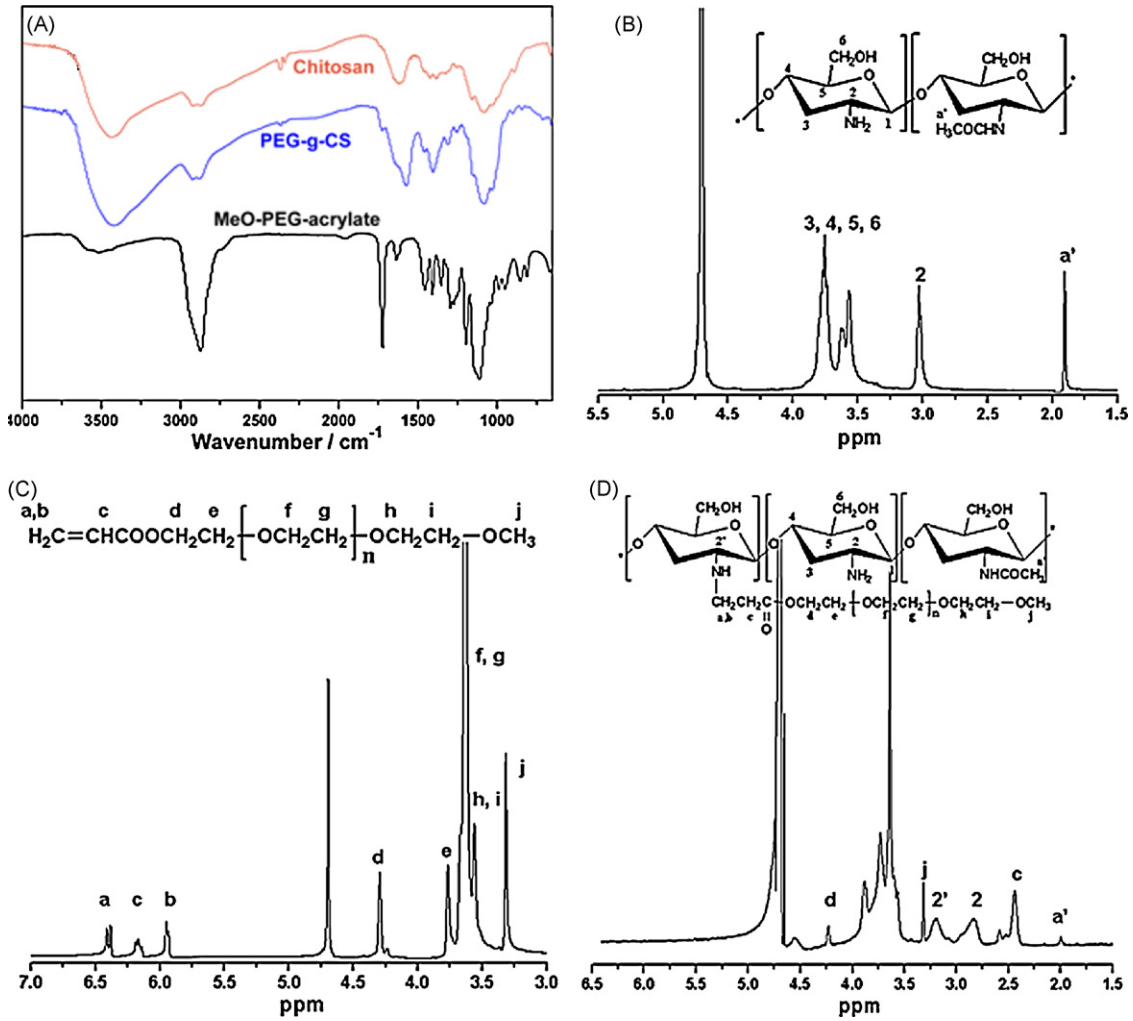

Fig. 1. (A) FT-IR spectra of chitosan, PEG-g-CS and MeO-PEG-acrylate, (B) ^{1}H NMR spectra of chitosan, (C) ^{1}H NMR spectra of MeO-PEG-acrylate, and (D) ^{1}H NMR spectra of PEG-g-CS.

PEG-g-CS, chitosan and methoxy polyethylene glycol monoacrylate (MeO-PEG-acrylate) shown in Fig. 1(A) confirms the success of grafting PEG on chitosan. For chitosan, the broad peak at 3440 cm^{-1} could be attributed to the stretching vibrations of –NH_2 and –OH, as well as inter- and intra-molecular hydrogen bonding. The weak peak located at 2926 cm^{-1} is associated with –CH– stretch in chitosan. The characteristic peaks at 1650, 1595 and 1320 cm^{-1} assigned to amine I, amine II and amide absorption band of chitosan. The MeO-PEG-acrylate characteristic peaks appeared 2930, 1720 and 1635 cm^{-1}, assigned to –CH– stretching vibration, C=O asymmetrical and symmetrical stretching and –C=C stretching vibration. For PEG-g-CS, the peaks corresponding to the stretching vibration of hydroxyl, amino and amide groups of chitosan shifted slightly. A small peak at 1730 cm^{-1} associated with C=O acrylate group in PEG implied that MeO-PEG-acrylate was grafted onto chitosan. Besides, the absorbance intensity of –CH– stretching vibration at 2930 cm^{-1} increased slightly.

Fig. 1(B) shows the ^{1}H NMR spectrum of chitosan in D_3CCOOD/D_2O. A singlet at 1.90 ppm is assigned to –CH_3 of GlcNAc residue. The peak at 3.01 assigned to H_2 of GlcN and the multiplets from 3.50 to 3.80 attributed to H_3, H_4, H_5, and H_6 of GlcN and GlcNAc. The ^{1}H NMR spectrum of MeO-PEG-acrylate is illustrated in Fig. 1(C): 6.41 (CH_2=CH–COO), 6.17 (CH_2=C*H*–COO), 5.93 (CH_2=CH–COO), 4.29 (–COOCH_2–), 3.56–3.76 (–COOCH_2CH_2–, –OCH_2CH_2–, –CH_2CH_2OCH_3), 3.32 (–OCH_3). For PEG-g-CS, in comparison with chitosan, peaks correspond to –COOCH_2CH_2– and –NH–CH_2CH_2–COO– appeared at 4.23 ppm and 2.40 ppm, respectively. The sharp signals at 3.32 ppm was assigned to –OCH_3 of PEG units. The peaks of PEG methylene were overlapped with those of H_3, H_4, H_5 and H_6 of glucosamine units. The DS value was evaluated by the relative peak intensities between –COOCH_2CH_2– (4.23 ppm) and –CH_3 (2.00 ppm) of GlcNAc residue in chitosan and calculated by the following formula:

$$\mathrm{DS} = \frac{3I_{4.23} \times (1 - \mathrm{DD})}{2I_{2.00}}$$

where I represents the integration area of the peak corresponding to the subscript of ^{1}H NMR ppm, DD represents the degree of deacetylation of chitosan (about 88%). The calculated DS for PEG-g-CS is about 40%.

3.3. Electrospun nanofibers characterization

3.3.1. SEM

Fig. 2 shows the scanning electron microscopy (SEM) images of nanofibers spun from aqueous solutions with different PEG-g-CS/PEO mass ratio. We attempted to electrospin of solution of pure PEG-g-CS by regulating the concentration of PEG-g-CS, but failed to obtain fibers. Pure PEG-g-CS itself was difficult to electrospun. To obtain nanofibers, PEO was selected as suitable partner

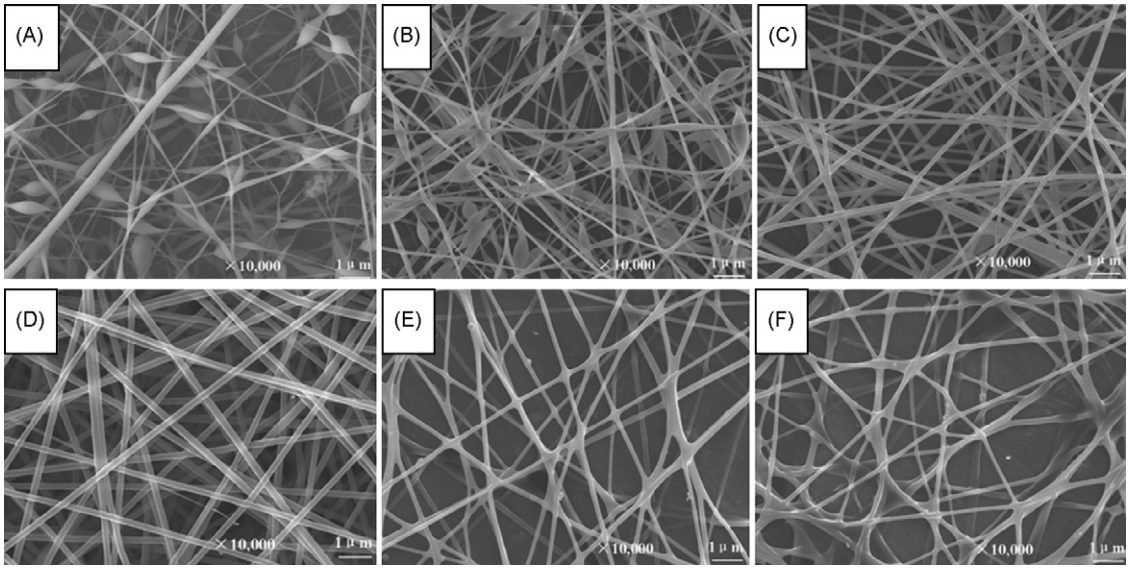

Fig. 2. SEM photographs of blended nanofibers with different mass ratios PEG-g-CS/PEO = 12/1 (A), 8/1 (B), 4/1 (C), 2/1 (D), 1/1 (E), and 1/2 (F).

for fabrication of nanofibers. When a small portion of PEO was mixed with PEG-g-CS (PEG-g-CS/PEO = 12/1), cylindrical fibers with considerable amount of elongated beads were deposited on the collector. When the mass ratio of PEG-g-CS/PEO reduced to 8/1, nanofibers with several beads could be obtained. When the mass ratio of PEG-g-CS/PEO ranged from 4/1 to 1/2, uniform ultrafine fibers with average diameter around 130–150 nm could be prepared as Fig. 2(C)–(F).

Table 1 shows the properties of blend electrospinning solutions with different PEG-g-CS/PEO mass ratios. The conductivity of PEG-g-CS/PEO mixed solutions reduced from 724 to 273 $\mu S\,cm^{-1}$ with the decrease of PEG-g-CS ratio, because PEG-g-CS is a polyelectrolyte while PEO is non-ionogenic polymer. The surface tension of mixture ranged from 48.8 to 61.9 mN/m, as shown in Table 1.

3.3.2. Stability of crosslinked electrospun nanofibers

All PEG-g-CS/PEO binary fibrous mats dissolved in water instantly because both PEG-g-CS and PEO are water-soluble polymers. To render them insoluble in water, glutaraldehyde crosslinked nanofibers were investigated. Fig. 3 shows the SEM images of glutaraldehyde crosslinked fibrous membranes after being immersed in water for 1 and 48 h. After crosslinked, the nanofibers were water resistant and could maintain fiber morphology even they immersed in water up to 48 h. Water resistance of nanofibers is desired for their biomedical application, while the biodegradability of chitosan also offers advantages for use in the long run.

Diameter distribution of the nanofibers before and after immersion in water is presented in Fig. 3(E)–(G). As the crosslinked nanofibers immersed in water, the average diameter of blend nanofibers increased obviously. Average fiber diameters of the original and crosslinked nanofibers were determined to be 130, 190 nm (immersed in water for 1 h) and 200 nm (immersed n in water for 48 h), respectively, indicating that the diameter of crosslinked nanofibers increased after immersed in water. Moreover, Fig. 3 also indicates that the fibers surface roughness increased after immersion in water for a long period.

3.3.3. Resistance to organic solvent

In order to characterize the organic solvent resistance of nanofibers, the samples were immersed in ethanol. After immersion in ethanol for 2 or 48 h, the fibers expanded in sizes but did not lost the original cylindrical form. Some separated adjacent fibers adhered to each other and merged into bundles at crosslinked-over regions. Fig. 4 compares the nanofibers prepared by PEG-g-CS/PEO (4/1) before (A and B) and after immersion in ethanol for 2 h (C and D) and 48 h (E and F). After immersion for 2 h, the nanofibers presented similar shape and morphology with small expansion in sized and adhesion. However, after 48 h, serious adhesion and swelling occurred as illustrated in Fig. 4(C) and (D). PEO can dissolve in both water and organic solvent such as ethanol, while PEG-g-CS as a water-soluble chitosan derivative cannot dissolve in ethanol. Besides, the original nanofibers were smooth and straight while the treated nanofibers showed curved and rough morphologies. Fig. 4(G)–(I) compares the diameter distribution of nanofibers before and after immersion in ethanol. The fiber diameters increased obviously after immersion in ethanol.

3.3.4. XRD and DSC analysis

Fig. 5(A) illustrates XRD patterns of chitosan and PEG-g-CS. Chitosan is a semi-crystal polymer and present typical peaks

Table 1
Mass ratios and properties of various electrospinning solutions.

Sample code	PEG-g-CS/PEO mass ratio	Conductivity ($\mu S\,cm^{-1}$)	Surface tension (mN/m)	Morphology
A	12/1	724	48.8	Nanofibers with many beads
B	8/1	692	49.0	Nanofibers with few beads
C	4/1	655	55.7	Nanofibers
D	2/1	503	56.0	Nanofibers
E	1/1	377	56.7	Nanofibers
F	1/2	273	61.9	Nanofibers

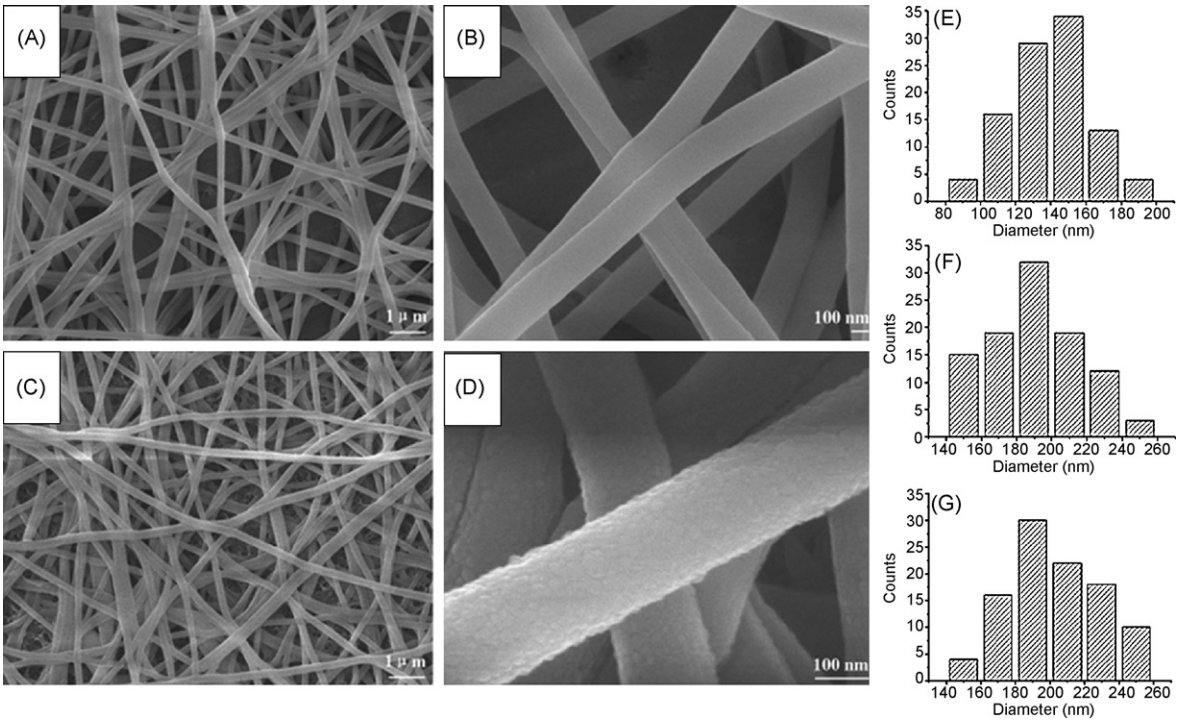

Fig. 3. (A–D) SEM images of crosslinked electrospun nanofibers after immersion in water for 1 h (A, B) and 48 h (C, D). (E–G) Diameter distribution of nanofibers before (E) and after immersion in water for 1 h (F) and 48 h (G).

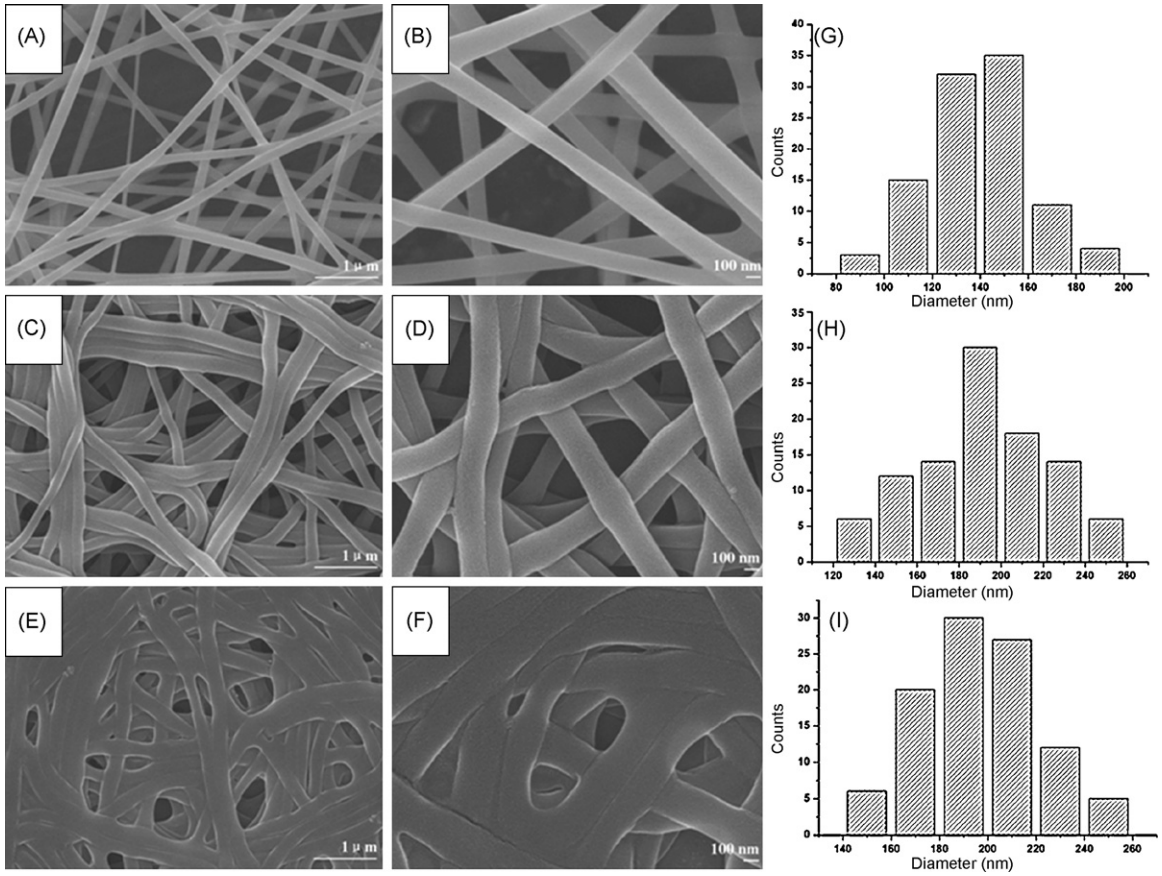

Fig. 4. (A–F) SEM images of electrospun nanofibers before (A, B) and after immersion in ethanol for 2 h (C, D) and 48 h (E, F). (G–I) Diameter distribution of nanofibers before (G) and after immersion in ethanol for 1 h (H) and 48 h (I).

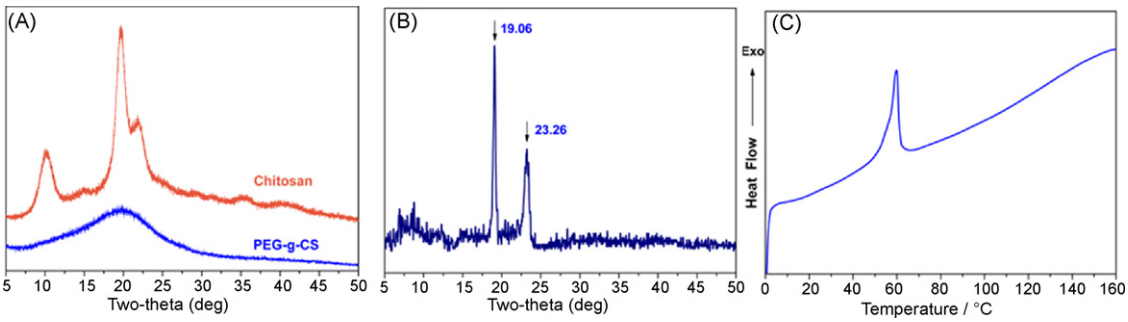

Fig. 5. (A) XRD patterns of chitosan and PEG-g-CS, (B) XRD pattern of PEG-g-CS/PEO (2/1) nanofibrous membranes, and (C) DSC curves of PEG-g-CS/PEO (2/1) nanofibrous membranes.

at 10.3° and 19.0° corresponded to crystal form I and form II, respectively (Ma et al., 2008). The reflection peaks of PEG-g-CS disappeared, implying amorphous state of PEG-g-CS. The reason for amorphous is the presence of PEG residues, which hinder the formation of inter- and intra-molecules hydrogen bonds after chemical modification. Fig. 5(B) presents XRD pattern of PEG-g-CS/PEO (2/1) blend nanofibers. There were two typical peaks at $2\theta = 19.06°$ and 23.26°, indicating the crystallization state of PEO competent. DSC result of PEG-g-CS/PEO (2/1) nanofibers also confirmed the crystallization of PEO, as shown in Fig. 5(C). However, the calculated melting enthalpy of PEO component in fibrous mat is much lower than PEO powder. This indicated that the crystalline microstructure of electrospun fibers did not develop well. The majority of the chains were in the non-crystalline state due to the rapid solidification process of stretched chains during electrospinning.

According to the literature, PEO powder showed strong reflection at 19.0° and 23.2°, corresponded to crystal planes of 120 and 112. After electrospun with PEG-g-CS, the reflection of PEO at 23° decayed obviously. During the electrospinning process, PEO component crystals of nanofibers were orientated for the tension effect. Moreover, the crystallinity degree of PEO decreased by the electrospinning process for the rapid evaporation of solvent. Furthermore, the PEG-g-CS in the solution would influence the crystal formation because of polymer chain entanglement, especially considering the chitosan derivative containing PEG units.

3.3.5. TEM

Fig. 6 shows the TEM micrographs of electrospun nanofibers with different PEG-g-CS/PEO mass ratio. When the mass ratio was 8/1, only fiber with elongated beads was observed. While the mass ratio of PEG-g-CS/PEO was descent to 4/1, imperfect core–shell structure fiber arose in nanofibers and the boundary between core and shell was ambiguous. Distinct core–shell structured fibers with obvious boundary could be found when the PEG-g-CS/PEO ratio decreased to 2/1 and 1/1. Increasing the amount of PEO in homogeneous solution could improve ratio of crystalline component and lead to fast phase separation during electrospinning process. The formation of core–shell structure might be due to the phase separation of the ternary system as explained in our previous paper (Zhang et al., 2009). The XRD and DSC results have demonstrated that PEO crystallized during the electrospinning process, while PEG-g-CS was amorphous.

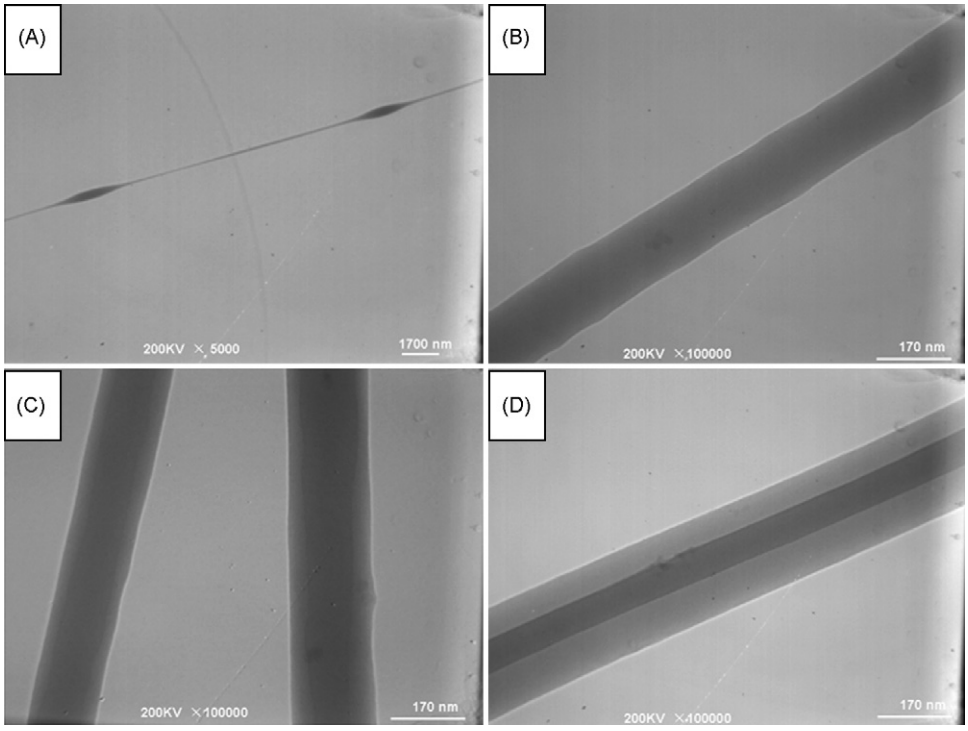

Fig. 6. TEM of electrospun nanofibers PEG-g-CS/PEO = 8/1 (A), 4/1 (B), 2/1 (C), and 1/1 (D).

4. Conclusions

In this study, PEG-g-CS with improved water solubility was prepared and the nanofibers based on PEG-g-CS blended with PEO were fabricated by electrospinning. Although electrospinning from aqueous solution of PEG-g-chitosan was unsuccessful, PEG-g-CS blended with a small amount of PEO could be electrospun into nanofibers without acids or organic solvents. Crosslinked nanofibers could maintain morphologies in water environment and therefore have the potential as tissue engineering scaffold and wound dressing. DSC and XRD results indicated the crystal state of PEO component. Core–shell structures have been found in nanofibers with certain PEG-g-CS/PEO mass ratio. The PEG-g-CS/PEO blended nanofibrous mats have the potential to be used as tissue engineering scaffolds, wound dressing and drug carriers in biomedical fields.

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