Preparation of Electrospun Polycaprolactone Nanofibers with Water-Soluble Eggshell Membrane and Catechin

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Received 25 March 2011; accepted 25 August 2011
DOI 10.1002/app.35538
Published online 6 December 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The process of incorporating water-soluble eggshell membrane (S-ESM) into polycaprolactone (PCL) electrospun nanofibers was investigated using the interaction between S-ESM and catechin. Electrospinning of the nontoxic natural catechin with PCL was examined, and S-ESM was introduced into the resulting PCL/catechin nanofibers through hydrogen bonding. S-ESM was added into PCL/catechin electrospun fibers by immersing the as-spun fibers in an S-ESM solution that was prepared by dissolving S-ESM powder in water with a dimethylformamide cosolvent. Morphological observation suggested that S-ESM was incorporated with catechin and formed S-ESM/catechin nanoparticles, distributed in the nanofiber webs. Analysis of Fourier transform infrared spectra indicated that hydrogen bonding interactions were generated between PCL and catechin as well as between S-ESM and catechin. Water contact angle tests suggested that the presence of S-ESM/catechin improved the wettability of PCL nanofiber webs. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: E83–E90, 2012

Key words: nanofiber; membranes; polyesters; soluble-eggshell membrane; catechin

INTRODUCTION

The health benefit of natural protein resource, such as silk fibroin, collagen, gelatin, and chitosan, has attracted much attention due to their biocompatibility and biodegradability.[1,2] To date, the natural proteins have been widely used as cosmetics, food additives, and medical materials for human health.[3] As for the specific application as medical materials, the natural protein materials are conventionally processed into fiber or film forms which could be used as wound dressing for wound healing. Electrospinning has been investigated as an effective technique for the production of nano-scale fibers. The nanofibers obtained from electrospinning consist of a high surface area to volume ratio resulting in various unique physical and chemical properties favoring for many applications.[4] The medical applications of protein-based nanofibers have been extensively discussed in the past decades. Huang et al.[5,6] first reported the electrospinning of collagen nanofibers as wound dressings. Thereafter, Park et al.[7] investigated the electrospinning condition of producing silk fibroin/chitosan nanofibers for wound healing application.

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Journal of Applied Polymer Science, Vol. 124, E83–E90 (2012)

Eggshell membrane (ESM), which contains collagen types I, V, and X, is a natural protein that is treated as waste. The ESM exhibits a fiber-like network structure resulting in adherence to skin texture and moisture retention properties, which are suitable characteristics for medical applications.[8–11] However, many disulfide bonds are present in the molecular structure of ESM, resulting in insolubility, which is a main obstacle to further application of ESM, and in particular, the production of ESM fibers. Rather than using natural ESM, water-soluble ESM (S-ESM) from natural hen ESM has been considered.[12–14]

To electrospin S-ESM from aqueous is hardly impossible due to the low viscosity, and the water-soluble biocompatible polymers, such as poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA), were considered to elecrospin with S-ESM for the S-ESM fiber formation.[15] In our previous study, electrospinning of S-ESM was investigated to determine if S-ESM fibers could be directly fabricated.[16] This electrospinning was performed by blending PEO and PVA with S-ESM to improve its low spinning processability. To try to maintain the fibrous structure in water using the soluble S-ESM/PEO and S-ESM/PVA as-spun fibers, the effect of incorporation of catechin with the as-spun fibers was examined. Catechin is a nontoxic, polyphenolic antioxidant plant metabolite that belongs to the flavonoid family, and that has been shown to possess

antibacterial, antioxidant, and other physiological functions.[17–20] Many studies have reported that the hydrogen bonding and hydrophobic interactions can be generated between protein and polyphenol;[21,22] therefore, the interaction between S-ESM and catechin was used to maintain the fibrous structure of S-ESM/PEO and S-ESM/PVA as-spun fibers in water. However, the result indicates that only a few S-ESM/catechin precipitate with fibrous structures were observed because some of the S-ESM failed to interact with catechin, and this free S-ESM was probably dissolved in water with PEO or PVA. Moreover, the obtained S-ESM/catechin precipitate was also brittle in nature. We therefore wished to determine if this process of fabrication of S-ESM nanofibers might be improved by adding S-ESM into a carrier electrospun polymeric nanofiber. For this purpose, polycaprolactone (PCL) was chosen as the base material of the polymer.

PCL is an aliphatic polyester that has been widely considered as a candidate material for medical application due to its excellent mechanical properties, biodegradability, and biocompatibility.[23–27] Several processes have been reported for incorporating S-ESM with aliphatic polymers. In one of these processes, the poly(D,L-lactic acid) PD-LLA membrane was first immersed in 5% aqueous hexafluoroisopropanol (HFIP) containing 3% (v/v) of the soluble eggshell membrane protein (SEP) and then 10% acetic acid containing 3% SEP was used to entrap the SEP molecules.[28] Jia et al.[29] reported the process to graft SEP onto the plasma treated PCL nanofibers. The results indicate a better cell attachment comparing to the pristine nanofibers. To explore an effective and simple process to add S-ESM into electrospun PCL nanofibers, catechin is considered to be used. Zhu et al.[30] reported that an intermolecular hydrogen bonding interaction was generated between the ester carbonyl groups of the polyesters and the phenol groups of catechin. Li et al.[31] confirmed the specific hydrogen bonding between PCL and phenol in various blend ratios of PCL/dihydric phenol films by Fourier transform infrared (FTIR). According to these results, as well as those of our previous study, it is possible to incorporate S-ESM with PCL nanofibers through the hydrogen bonding interaction between S-ESM/catechin and PCL/catechin.

In this study, we aim to develop a simple process to prepare PCL-based S-ESM/catechin nanofiber webs. First, a process to prepare electrospun PCL nanofibers by blending PCL with catechin was developed and analyzed. Second, a simple method of adding S-ESM into the electrospun PCL/catechin nanofiber webs was examined to generate the hydrogen bonding interaction between S-ESM and catechin. The hypothesis was that the added catechin might encapsulate S-ESM with the PCL nanofibers, and then PCL-based S-ESM/catechin nanofibers could combine all the benefit properties of materials and have potential to be used as wound dressing in medical field.

TABLE I
Electrospinning Dopes and Spinning Conditions

Electrospinning dopes		Electrospinning condition	
PCL : Catechin (w/w)	Total solutes concentration (wt %)	Voltage (kV)	Feed rate (mL/h)
100 : 0	8	9	0.10
95 : 5	8.4	9	0.08
90 : 10	8.8	8	0.10
85 : 15	9.3	8	0.08
80 : 20	9.8	8	0.10
75 : 25	10.4	6	0.08

Ambient condition: 21°C, 40% (R.H).

EXPERIMENTAL

Materials

S-ESM powder ($Mw = 6000$) and catechin powder (P70-A, $Mw = 420$) were provided by Idemitsu Technofine Co., Chiba, Japan. PCL ($Mn = 70{,}000$–$90{,}000$) was purchased from Sigma-Aldrich Corp., St. Louis, MO. Dichloromethane (CH_2Cl_2) and dimethylformamide (DMF) were used as a solvent for PCL and were purchased from Nacalai Tesque, Kyoto, Japan.

Preparation of PCL/catechin spinning dopes

The PCL pellets were dissolved in CH_2Cl_2/DMF cosolvents (CH_2Cl_2/DMF = 60 : 40, w/w) with 8 wt % concentration. The PCL/catechin spinning dopes, with different weight ratios of PCL : catechin, were prepared by adding catechin powder into the 8 wt % PCL solution, and then stirring for 24 h as described in Table I. The dynamic viscosity of PCL and PCL/catechin dopes were measured by viscosimeter (model: RVDV-II +P CP, Brookfield, Massachusetts, USA) at 25°C.

Electrospinning

The electrospinning device was set up by placing a 3 mL syringe, capped with a 27-G size needle, in a syringe pump. A copper collection plate (8 × 8 cm) covered with aluminum foil was used as the collector. Distance between collector and needle is 20 cm. Random fibers were formed on the collector and were dried in a desiccator for 24 h. The conditions used for electrospinning are also listed in Table I.

Addition of S-ESM to PCL/catechin as-spun fibers

When pure S-ESM and catechin were mixed in water, strong precipitation occurred as a result of

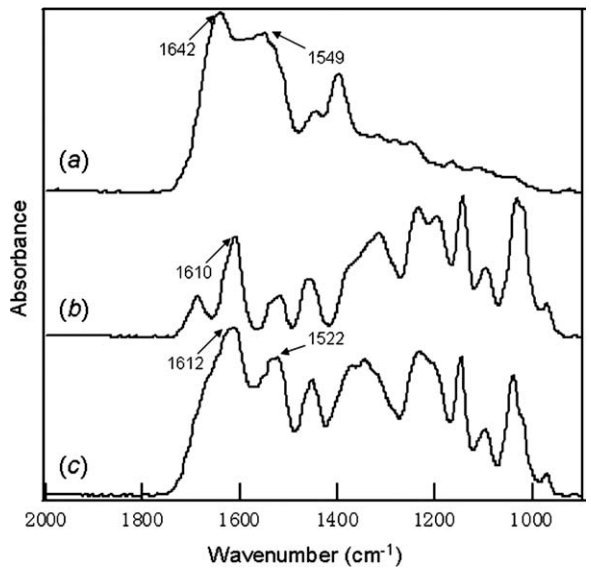

Figure 1 FTIR spectra of (a) S-ESM, (b) catechin, and (c) S-ESM and catechin.

the hydrogen bonding interaction between S-ESM and catechin. This interaction was the key property required for addition of S-ESM into the PCL/catechin as-spun fibers. S-ESM was added into the PCL/catechin as-spun fibers as an S-ESM solution. This S-ESM solution was prepared by dissolving S-ESM powder in a water/DMF (95/5, w/w) cosolvent at a concentration of 9.5 wt %**.** DMF was used for this purpose so that S-ESM could diffuse into the PCL/catechin as-spun fibers, as there was a possibility that the PCL/catechin as-spun fiber could swell in the S-ESM solution. The PCL/catechin as-spun fibers deposited on aluminum foil (1 × 1 cm^2) were immersed in S-ESM solution for 3 days, and then vacuum dried under flat state in a desiccator at room temperature.

Characterization of the nanofibers

Morphology observation

For the observation of the electrospun nanofibers, the samples were sputter-coated with Au/Pd and examined with a field emission scanning electron microscopy (FE-SEM, Hitachi S4200). The diameter of individual electrospun nanofibers was evaluated using ImageJ.

FTIR analysis

Attenuated total reflection FTIR spectroscopy (Perkin Elmer Spectrum GX) was used to investigate the chemical composition of the sample at each stage of the process. The samples were examined using 8 cm^{-1} resolution over a range of 4000–700 cm^{-1}. Each measurement was composed of an average of 16 scans.

Water contact angle test

The water contact angle (WCA) was measured using a contact angle tester (CA-S150, KYOWA Interface Science Co., Japan). Deionized water (4 μL) was dropped on the surface of electrospun nanofiber webs at room temperature. The change in the contact angle was recorded and measured using ImageJ software.

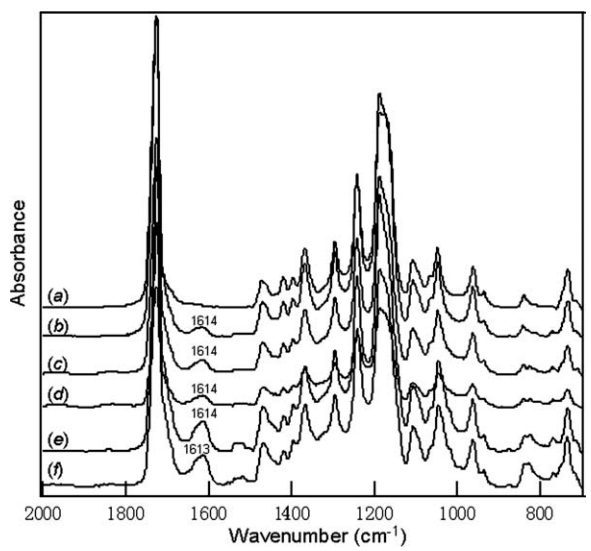

Figure 2 FTIR spectra of as-spun fibers (a) pure PCL, (b) PCL : catechin = 95 : 5, (c) PCL : catechin = 90 : 10, (d) PCL : catechin = 85 : 15, (e) PCL : catechin = 80 : 20, and (f) PCL : catechin = 75 : 25.

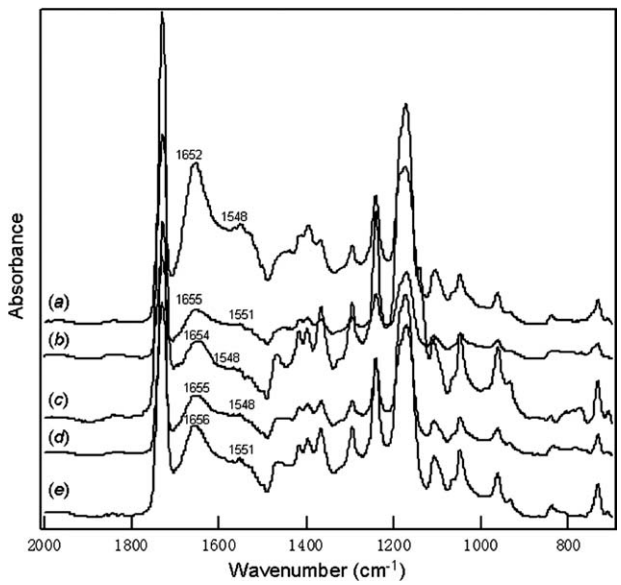

Figure 3 FTIR spectra of S-ESM treated as-spun fibers made from (a) pure PCL, (b) PCL : catechin = 95 : 5, (c) PCL : catechin = 90 : 10, (d) PCL : catechin = 85 : 15, and (e) PCL : catechin = 80 : 20.

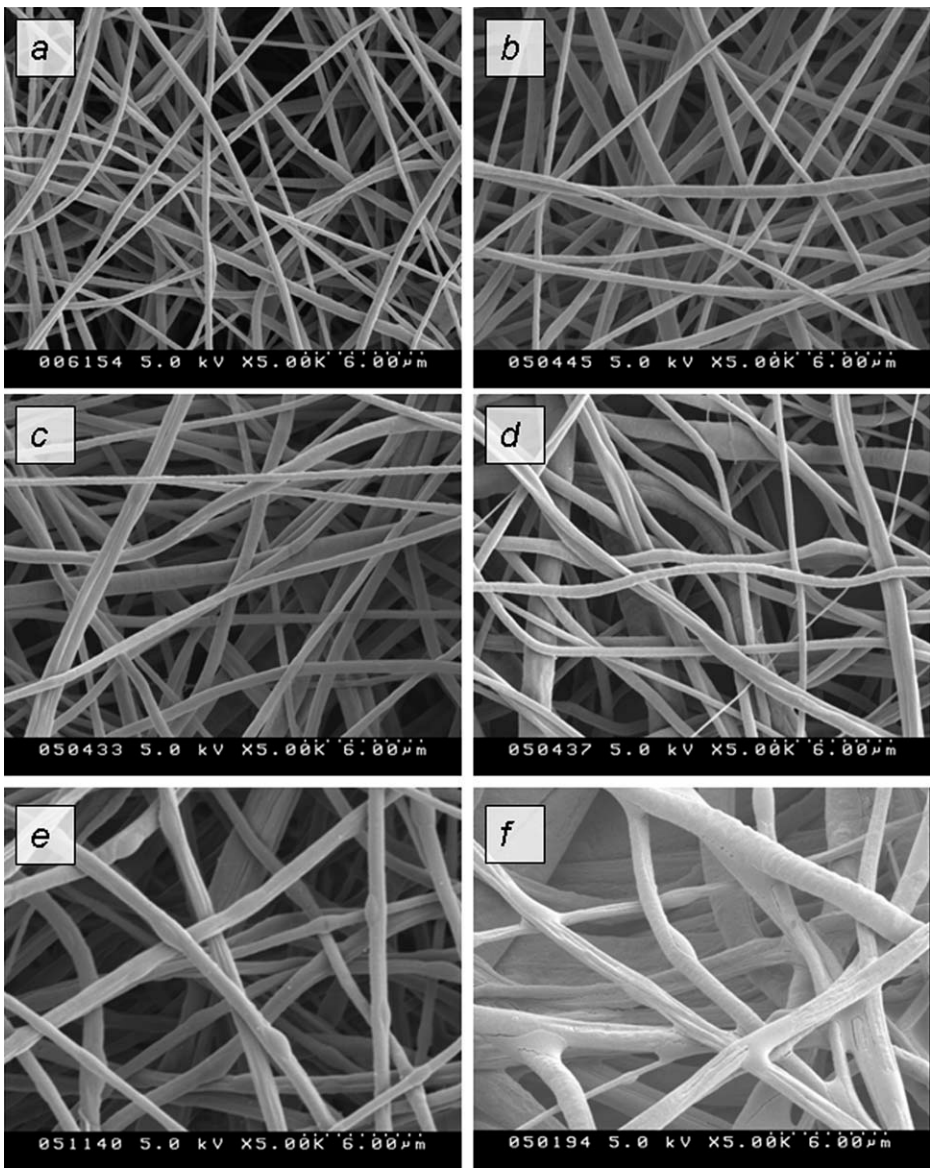

Figure 4 FE-SEM micrographs of as-spun fibers made from (a) pure PCL, (b) PCL : catechin = 95 : 5, (c) PCL : catechin = 90 : 10, (d) PCL : catechin = 85 : 15, (e) PCL : catechin = 80 : 20, and (f) PCL : catechin = 75 : 25.

RESULTS AND DISCUSSION

FTIR spectroscopy

S-ESM and catechin powders

The FTIR spectra of the S-ESM powder, the catechin powder, and the precipitated particles of S-ESM/catechin (50 : 50, w/w) are shown in Figure 1. In our previous study, we showed that, after blending with catechin, the characteristic peaks of carbonyl (amide I) and N—H bending (amide II) bands in S-ESM were shifted to 1612 and 1522 cm^{-1} from 1642 and 1549 cm^{-1}, respectively.[11] The band at 1612 cm^{-1} could be related to hydrogen-bonded carbonyl vibration implying the formation of hydrogen bonds between S-ESM and catechin.

PCL and PCL/catechin as-spun fibers

FTIR spectra of pure PCL and PCL/catechin as-spun fibers at catechin blend ratios of 5 wt % to 30 wt % are shown in Figure 2. A new peak appeared at around 1614 cm^{-1} following catechin addition, which was higher than the peak of C=C stretching in the aromatic rings of catechin alone at 1610 cm^{-1}. This new peak was attributed to the hydrogen-bonded carbonyl groups formed between the ester carbonyl group in PCL and the hydroxyl group in catechin. This result also indicated that the absorbance of hydrogen-bonded carbonyl groups slightly increased with increasing catechin content of the as-spun fibers. Zhu et al.[30] also observed that the relative absorbance of hydrogen-bonded carbonyl

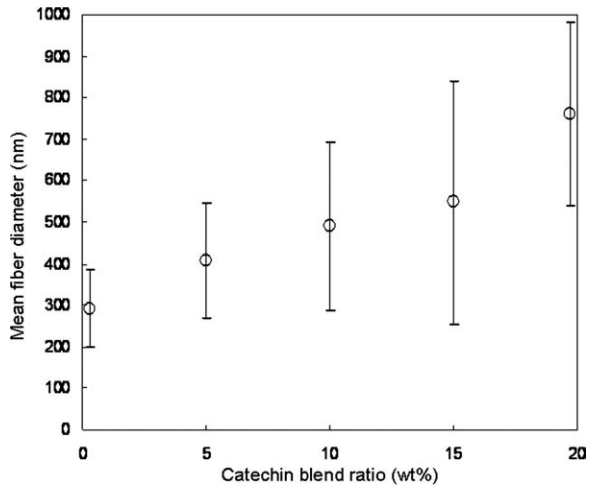

Figure 5 Mean fiber diameter of PCL/catechin blend nanofibers.

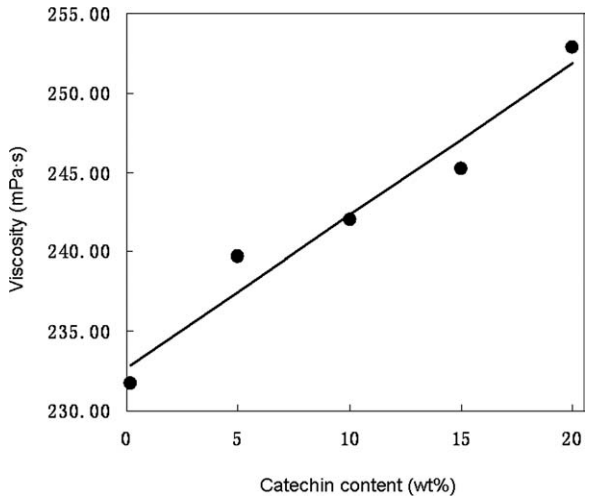

Figure 6 The effect of catechin content on the viscosity of PCL/catechin solutions.

vibration increased with increasing catechin content of polyester/catechin blend films. These FTIR results imply that the catechin content in a PCL/catechin spinning solution affects the hydrogen bonding interaction between PCL and catechin resulting in different PCL/catechin fiber formations.

PCL/catechin as-spun fibers after S-ESM treatment

The FTIR spectra of pure PCL and PCL/catechin as-spun fibers after S-ESM treatment are shown in Figure 3. The characteristic peaks of carbonyl (amide I) and N—H bending (amide II) bands of S-ESM were observed for all the samples after S-ESM treatment,

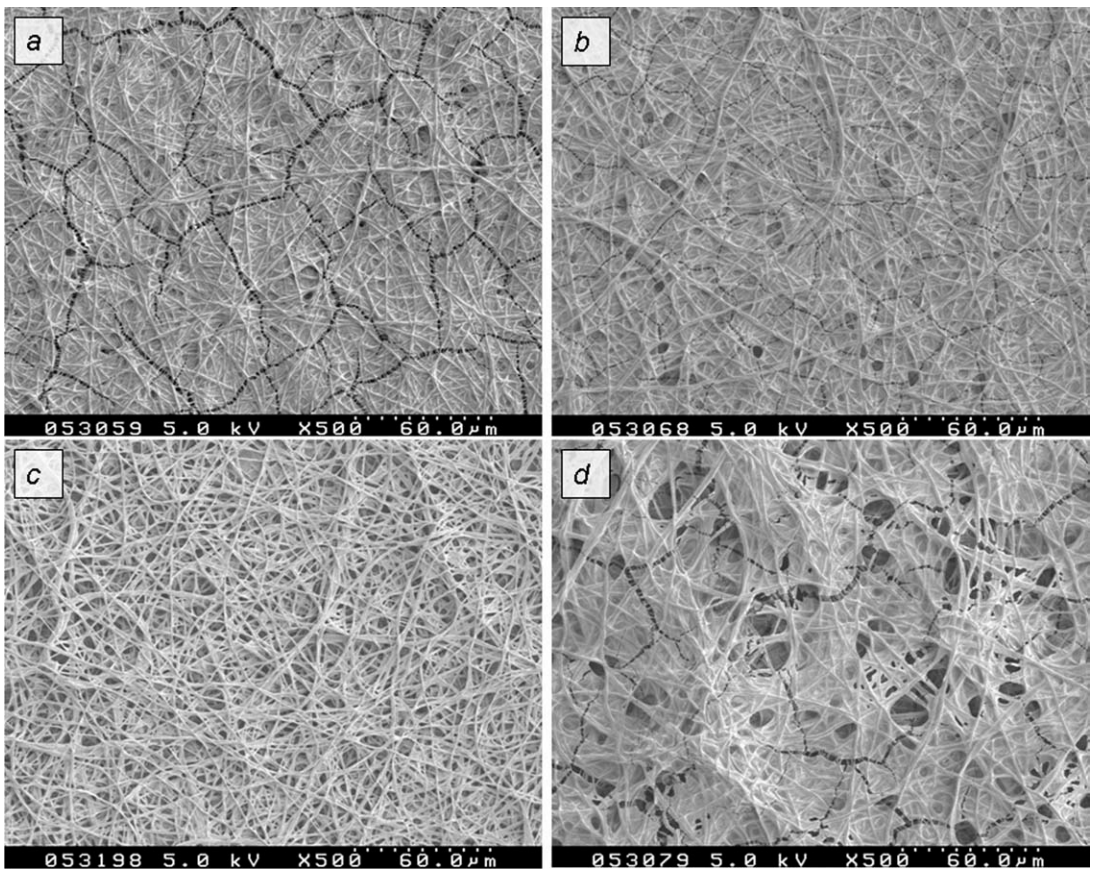

Figure 7 FE-SEM micrographs (low magnification) of S-ESM treated fibers: (a) pure PCL, (b) PCL : catechin = 95 : 5, (c) PCL : catechin = 90 : 10, and (d) PCL : catechin = 80 : 20.

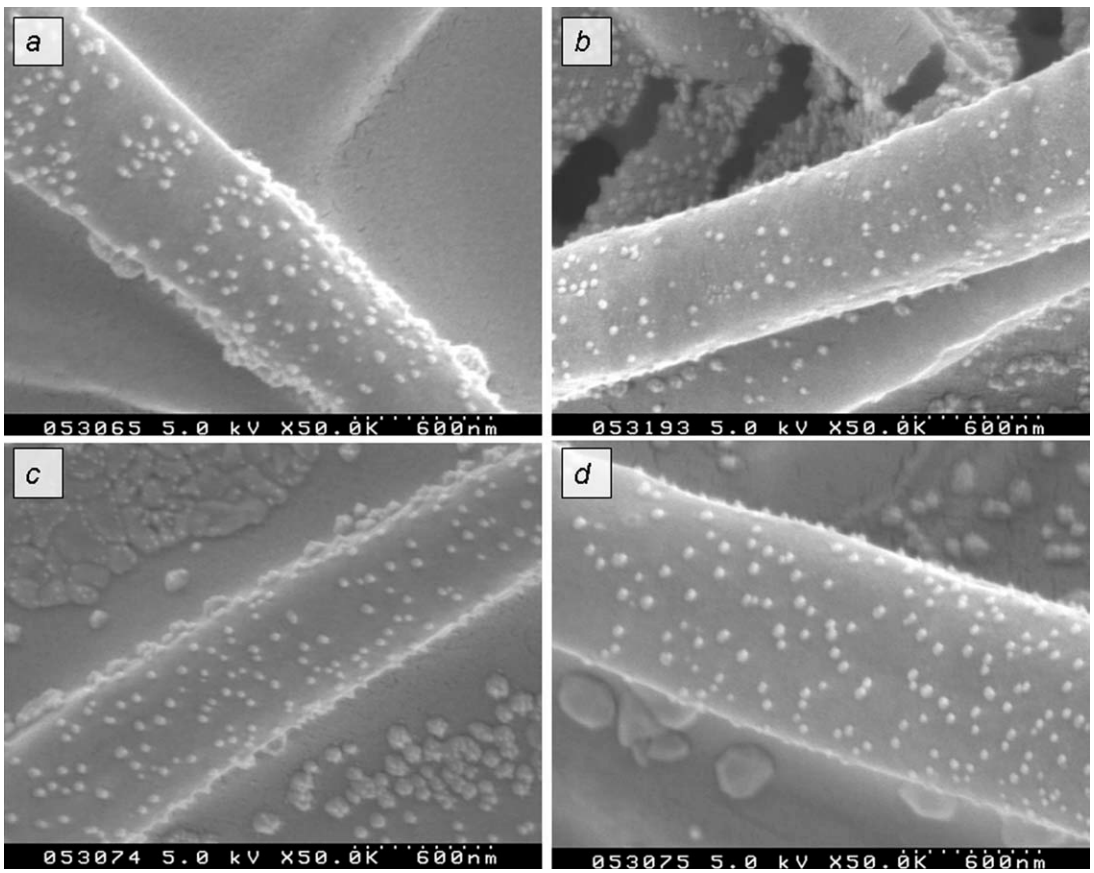

Figure 8 FE-SEM micrographs (high magnification) of S-ESM treated fibers: (a) PCL : catechin = 95 : 5, (b) PCL : catechin = 90 : 10, (c) PCL : catechin = 85 : 15, and (d) PCL : catechin = 80 : 20.

due to the presence of S-ESM in the nanofiber webs. In addition, a shift of the amide I band to a higher frequency occurred, which occurred as a result of the increase in the carbonyl band density. These results also indicate that the carbonyl vibration region of S-ESM treated PCL/catechin was broadened compared with that of pure PCL as-spun fibers after S-ESM treatment. These data imply the existence of hydrogen bonding interactions between PCL/catechin and catechin/S-ESM, which corroborates the FTIR results in Figures 1 and 2.

Morphology

PCL/catechin as-spun fibers

The effect of catechin weight ratios of blended dopes on fiber morphology was investigated. The morphology of PCL/catechin blend fibers is shown in Figure 4. Fine uniform blend fibers were electrospun at blend ratios of PCL : catechin of 95 : 5 and 90 : 10 [Fig. 4(b,c)]. When the percentage of catechin was increased to 15 or 20 wt % in the spinning solution, the resulting fiber diameter was inhomogeneous and the fiber surface was not smooth [Fig. 4(d,e)] compared with fibers prepared using a low catechin content [Fig. 4(b,c)]. When solutions composed of PCL/catechin at ratios of 75 : 25 [Fig. 4(f)] was electrospun, solution droplets and only a few fibers were observed on the collector. When the percentage of catechin was greater than 25 wt%, no fiber was formed by electrospinning. Thus, the solution spinning-ability decreased as the catechin content increased. The mean fiber diameter of PCL/catechin as-spun fibers with the catechin content from 5 wt % to 20 wt % is shown in Figure 5. An increase in the mean fiber diameter was observed with increasing catechin content. Many studies have been reported that increasing solution viscosity associated with the production of large diameter fibers.[32,33] The relationship between viscosity and catechin content of spinning solutions was shown in Figure 6. The viscosity of solution was increased with increasing the catechin content, and resulted in an increase of fiber diameter. The increase of PCL/catechin viscosity could be due to the increase of solution concentration as well as the hydrogen bonding interactions between PCL and catechin. According to the morphological observations, a catechin content ranging between 5 wt % and 20 wt % resulted in the formation of uniform PCL/catechin nanofiber webs that were suitable for treatment with S-ESM.

PCL/catechin nanofibers after S-ESM treatment

The changes that occurred in the morphology of pure PCL and PCL/catechin nanofibers after treatment with S-ESM at low magnification are shown in Figure 7. A membrane-like morphology was observed for the S-ESM treated PCL and PCL : catechin = 95 : 5 nanofibers [Fig. 7(a,b)]. When sample was taken out from the S-ESM solution, residual S-ESM solution formed a membrane between the fibers after vacuum drying. When the residual S-ESM solution was removed after the taking out process, less membrane was formed between the fibers as shown in the PCL : catechin=90 : 10 and 80 : 20 samples [Fig. 7(c,d)]. In Figure 8, the fiber surface of S-ESM treated samples was shown at high magnification. The nanoparticles with diameters of less than 60 nm were observed on the fiber surface for all blend ratios of PCL/catechin fibers. These nanoparticles could be considered as precipitates formed by catechin and S-ESM though hydrogen bonding interaction, because no particle appeared on PCL alone that was treated with the S-ESM solution. It is assumed that the S-ESM diffuses into a PCL/catechin blend nanofiber and also that catechin oozes out from a fiber, following which S-ESM and catechin form particles on the fiber surface and between the fibers. The size of the nanoparticles was unrelated to the PCL : catechin blend ratio. These morphological observations show that, ultimately, S-ESM/catechin is presented on the PCL nanofibers as nanoparticles form.

The results of FTIR spectra and morphology observation show that, ultimately, S-ESM/catechin nanoparticles are present on the PCL nanofibers through the interaction between S-ESM and catechin. Catechin could be used as a suitable linking material for incorporating S-ESM with PCL nanofibers.

WCA test of nanofibers

The WCA of PCL, PCL/catechin, and S-ESM treated PCL/catechin fibers at both 1 s and 20 s after treatment is shown in Figure 9. The contact angle of pure electrospun PCL was almost a constant 126° during the test. Martins et al.[34] reported a contact angle of 130° for PCL nanofiber meshes. The result is close to this value. The PCL/catechin = 95 : 5 nanofiber webs showed an initial contact angle of 123°, and it slightly decreased after 20 s. When the PCL/catechin = 90 : 10 nanofiber webs were measured, the contact angle significant decreased to 35° from an initial angle of 86° after 20 s. The contact angle of PCL/catechin = 80 : 20 was lowest both in the initial and after 20 s comparing with other ratios. These results indicate the presence of catechin has effect on improving surface wettability of PCL nanofibers,

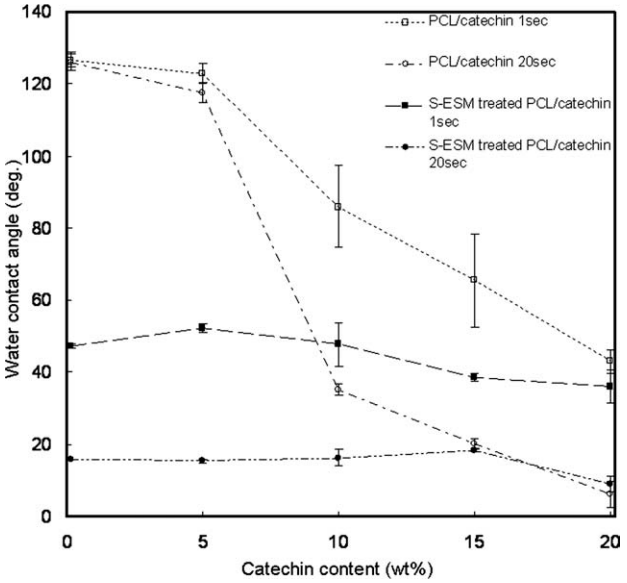

Figure 9 The WCA of nanofiber webs.

and the wettability was increased when increasing the catechin content in the nanofibers. The value of the contact angle of PCL/catechin/S-ESM nanofiber webs was significantly decreased compared with that of pure PCL and PCL/catechin nanofibers. The S-ESM and residual S-ESM membrane in the nanofibers (as shown in Fig. 4), absorb water, which makes the web surface more hydrophilic. These WCA results suggest the presence of catechin and S-ESM changed the wettability of PCL nanofibers.

CONCLUSIONS

In this study, the process of incorporating S-ESM into PCL electrospun nanofibers was examined to determine its effect on the generation of PCL-based S-ESM/catechin nanofibers. The nontoxic natural catechin was used for electrospinning with PCL, and for the introduction of S-ESM into the PCL nanofibers through hydrogen bonding. FTIR spectroscopy confirmed that a hydrogen bonding interaction occurred between PCL/catechin and catechin/S-ESM. The uniform PCL/catechin fibers were produced at blend ratios of PCL : catechin of 95 : 5, 90 : 10, 85 : 15, and 80 : 20. The results suggested that the catechin content had an effect on uniform fiber formation. S-ESM was incorporated into the PCL/catechin electrospun nanofibers by immersing the as-spun fibers in an S-ESM solution. S-ESM/catechin precipitates in nanoparticle form were formed on the surface of the PCL/catechin nanofibers following immersion of the S-ESM solution due to the interaction between S-ESM and catechin. No nanoparticle was formed after S-ESM treatment of pure PCL nanofibers. The residual S-ESM could form a membrane-like structure due to the immersion process. The WCA results suggested

the presence of catechin and S-ESM improved the wettability of PCL nanofibers. Further experiments are required improving the immersion process for the addition of S-ESM into PCL/catechin as-spun fibers to form PCL-based S-ESM/catechin nanofibers. The PCL-based nanofibers with S-ESM/catechin nanoparticles could have potential application as wound dressing in medical field.

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