

# Fabrication of Electrospun Eggshell Membrane Nanofibers by Treatment with Catechin

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**ABSTRACT:** Optimum processing conditions for the electrospinning of water-soluble eggshell membrane (S-ESM) were investigated. Two biocompatible polymers, poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA), were used to improve the processability of S-ESM for electrospinning in aqueous media. Uniform nanofibers with average diameters of 240 and 335 nm were successfully electrospun for blend ratios at S-ESM/PEO = 95 : 5 and S-ESM/PVA = 60 : 40 with a solutes concentration of 20 and 18 wt %, respectively. Insoluble S-ESM/PEO and S-

ESM/PVA fibers were achieved after treatment with catechin and required no toxic or synthetic additives. FTIR spectra indicated that the hydrogen bond formed between S-ESM and catechin was the main interaction that improved the insolubility of S-ESM/PEO and S-ESM/PVA nanofibers in water. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 2042–2049, 2010

**Key words:** crosslinking; fibers; nanotechnology; water-soluble eggshell membrane; catechin

## INTRODUCTION

Electrospinning of various natural protein-based materials, such as keratin, collagen, and silk fibroin has been widely investigated in recent years.<sup>1–3</sup> Protein-based electrospun nanofibers have a high surface area to volume ratio, unique physical properties, and biocompatibility, which make them suitable to mimic the scaffolds of human skin tissue in biomedical applications, such as wound dressings and cosmetic sheets. Organic solvents such as formic acid, hexafluoroacetone, and isopropanol are generally used for the electrospinning of protein-based materials<sup>4</sup> and could be toxic to native human skin tissue. Therefore, to improve the biocompatibility of protein-based nanofibers, electrospinning in aqueous media should be investigated for process improvement.

Eggshell membrane (ESM), which contains collagen types I, V, and X, is a common waste material in daily life. ESM exhibits the cell-producing action of amino acids, adherence to textured surfaces due to a network structure, appropriate moisture retention, and air permeability, which are suitable characteristics for medical applications.<sup>5</sup> ESM has been used as a media for biosorption, templates for forming ordered tube networks, and as a biodegradable bone regeneration inhibitor.<sup>6–8</sup> 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. Yi et al. obtained S-ESM protein by dissolving raw ESM powder and they reported that polyethylene (PE) and poly(vinyl alcohol) (PVA) films treated with S-ESM showed significant culturing of NIH3T3 cells compared with untreated films.<sup>9–11</sup> Electrospinning of S-ESM is difficult, due to its low molecular weight. Yi et al. reported that a blend of S-ESM protein with poly(ethylene oxide) (PEO) increased the viscosity of spinning solution (dope) used to produce fibers by electrospinning.<sup>12</sup> Kim et al. also obtained S-ESM protein/poly( $\epsilon$ -caprolactone) (PCL) blended nanofibers using a coaxial electrospinning process.<sup>13</sup>

Catechin is a polyphenolic antioxidant plant metabolite that belongs to the family of flavonoids, and it has been shown to possess antibacterial, antioxidant, and other physiological functions.<sup>14–17</sup> The interaction between protein and polyphenol is widely discussed in the food industry. For example, Siebert et al. reported that the amount of haze formation is determined by the concentration and ratio of protein and polyphenol.<sup>18</sup> It has also been reported that collagen protein interacts strongly with the catechin polyphenol through hydrogen bonding, and no major changes in the secondary structure of collagen occurred after treatment with catechin.<sup>19</sup>

In this study, S-ESM was chosen as the protein-based material for electrospinning in aqueous media. PEO and PVA were selected as supporting polymers

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**TABLE I**  
**The Electrospinning Condition of S-ESM/PEO and S-ESM/PVA Blend Dope**

	Blend ratio (w/w)	Solutes concentration (wt %)	Voltage (kV)	Feed rate (mL/h)	Distance (cm)	Fiber form
S-ESM	–	40	6–10	0.1–0.2	15–22	No fiber
S-ESM/PEO	97 : 3	20	8.5	0.1	15–22	Particles
	95 : 5	10	6.5	0.1	15	Beaded fiber
	95 : 5	15	6.5	0.1	15	Beaded fiber
	95 : 5	20	6.5	0.1	15	Fiber
	90 : 10	20	6.0	0.1	15	Fiber
	80 : 20	20	9.0	0.1	15	Fiber
S-ESM/PVA	90 : 10	16	10.5	0.1–0.2	15	No fiber
	80 : 20	18	10.5	0.2	15	Beaded fiber
	70 : 30	20	10.5	0.1	15	Fiber
	60 : 40	18	10.5	0.15	15	Fiber
	50 : 50	16	10.5	0.1	15	Fiber

for incorporation with S-ESM, because they have been shown to have good processability for blending with many natural materials favored for electrospinning. First, the optimum solution concentrations and blend ratios for the production of uniform nanofibers were investigated by electrospinning various S-ESM/PEO and S-ESM/PVA solutions. Secondly, various process conditions were examined to develop a method in which S-ESM nanofibers become insoluble through the interaction between S-ESM and catechin without toxic or synthetic additives. We hypothesized that S-ESM protein in an S-ESM/polymer blend fiber encapsulated by catechin may result in enhanced S-ESM stability in water. Our future goal is to fabricate insoluble regenerated ESM nanofibers with catechin in aqueous media.

## EXPERIMENTAL

### Materials

S-ESM powder ( $M_w = 6000$ , Idemitsu Technofine Co.) was used as a starting material. Catechin is a water-soluble powder (P70-A,  $M_w = 420$ , Idemitsu Technofine Co.) extracted from green tea. PEO ( $M_w = 900$  K, Sigma-Aldrich), and PVA (hydrolysis: 87–89 mol %, Kuraray Poval) were used as supporting polymers for blending with S-ESM. Ethanol (99.5%) was purchased from Nacalai Tesque.

### Electrospinning of S-ESM blend fibers

Spinning solutions were prepared by mixing 40 wt % S-ESM aqueous solution with 5 wt % PEO and 10 wt % PVA aqueous solutions respectively at various blend ratios (w/w), as shown in Table I. All mixtures were stirred at room temperature for 24 h. The electrospinning device was set up by placing a 3-mL syringe capped with a 22-G size needle in a syringe pump. A copper collection plate covered with aluminum foil was used as the collector. Random fibers

were formed on the collector, and then dried in a desiccator. The conditions used for electrospinning are listed in Table I.

### Addition of catechin and nanofiber solubility test

When both S-ESM and catechin were mixed in water, a strong precipitation occurred that could not be used as a spinning dope. This interaction between protein and catechin was the key property under examination in this study. Alternatively, catechin was added to the fibers as follows. Catechin solutions (10 and 40 wt %) were prepared using ethanol as a solvent. The electrospun nanofibers on aluminum foil ( $3 \times 1 \text{ cm}^2$ ) were immersed into the catechin/ethanol solution for different immersion times of 1, 3, and 7 days. The effect of the immersion time on the fiber morphology was examined by drying the catechin-treated fibers in a desiccator at room temperature and then immersing in water for 12 h to investigate the change in solubility.

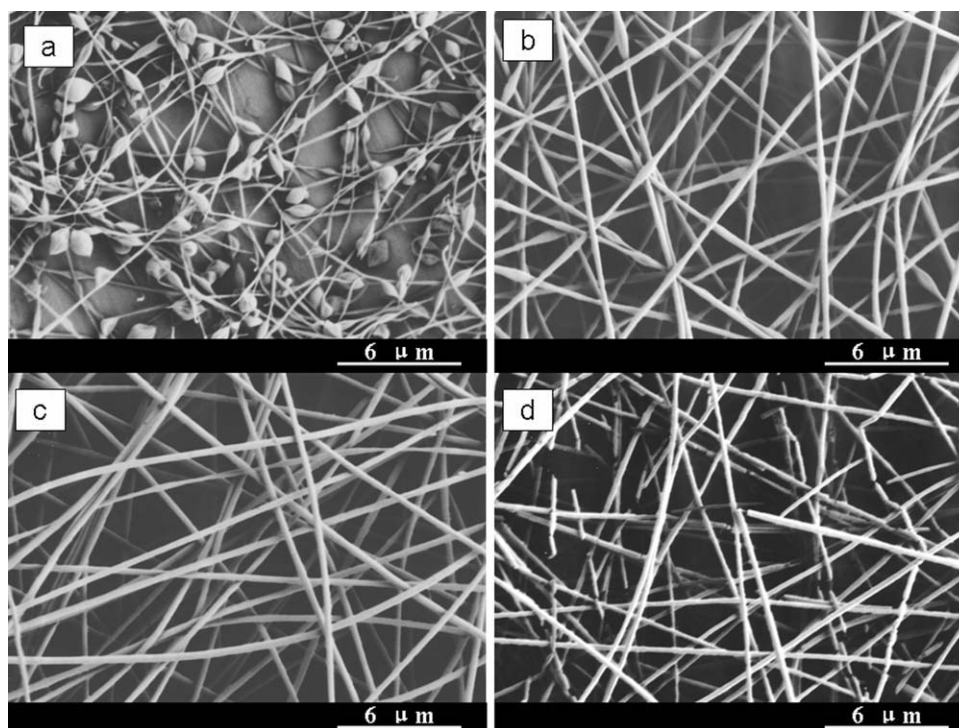
### Characterization

The morphology of gold-sputtered fibers was examined using field emission scanning electron microscopy (FE-SEM; Hitachi S4200). Fourier transform infrared spectroscopy (FTIR; PerkinElmer Spectrum GX) was used to examine the chemical composition of the fibers at each stage of the process.

## RESULTS AND DISCUSSION

### Morphology of as-spun fibers produced from S-ESM/PEO and S-ESM/PVA aqueous solutions

Electrospinning of the 40 wt % S-ESM aqueous solution was unsuccessful and no fibers were formed at any electric field or feed rate. The low molecular weight of S-ESM reveals its low viscosity and no



**Figure 1** FE-SEM micrographs of (a) S-ESM/PEO = 95 : 5 as-spun fibers with 15 wt % solutes concentration, (b) S-ESM/PEO = 95 : 5 as-spun fibers with 20 wt % solutes concentration, (c) S-ESM/PEO = 90 : 10 as-spun fibers with 20 wt % solutes concentration, and (d) S-ESM/PEO = 80 : 20 as-spun fibers with 20 wt % solutes concentration.

stable drop could be formed on the tip of needle for the formation of fibers. However, particle formation was observed for a blend ratio of S-ESM/PEO = 97 : 3. Figure 1 shows the morphology of as-spun S-ESM/PEO fibers at different blend ratios. When the blend ratio was 95 : 5, beaded fiber was obtained at solute concentrations of 10 and 15 wt % [Fig. 1(a)]. Fine uniform fibers [Fig. 1(b)] without beads were produced by increasing the solution concentration to 20 wt % at the same blend ratio. Figure 1(c,d) show that the morphology of as-spun fibers at blend ratios of S-ESM/PEO = 90 : 10 and 80 : 20 with 20 wt % solute concentration was not significantly different to the 95 : 5 as-spun fibers with the same solute concentration. A solute concentration of 20 wt % was required to produce uniform fibers without beads.

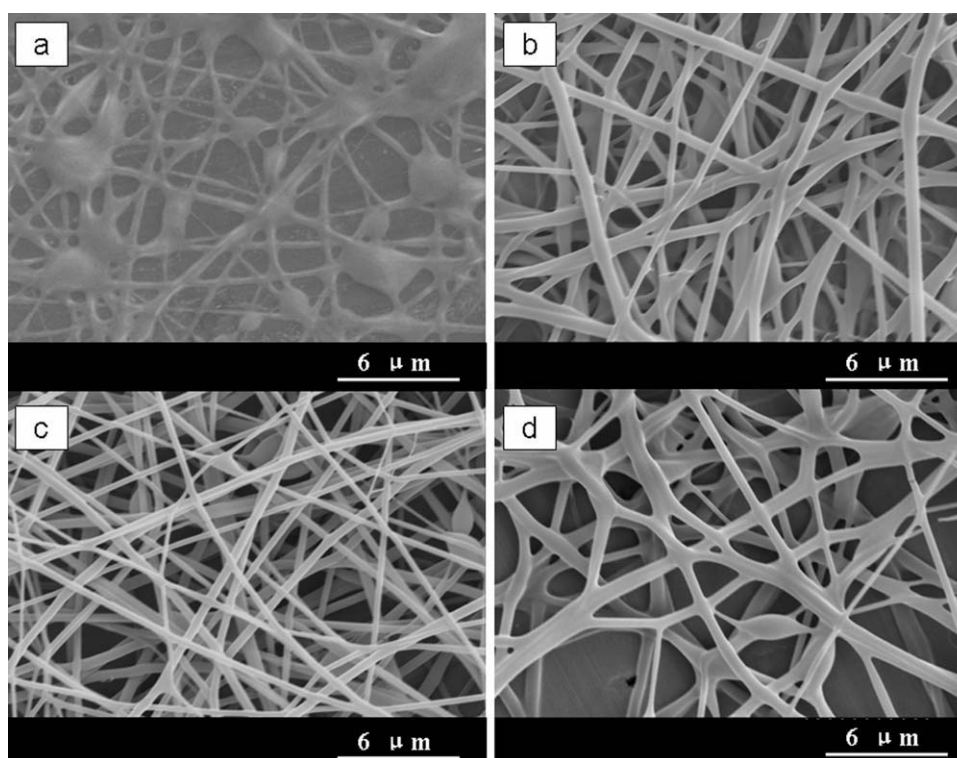
When PVA was used as the supporting polymer, no fibers were formed at a blend ratio of S-ESM/PVA = 90 : 10 under any spinning conditions. Beaded fibers were produced for the blend ratio of 80 : 20 with 18 wt % solute concentration [Fig. 2(a)]. Fibers were formed with solution droplets on the aluminum foil during electrospinning of the S-ESM/PVA = 70 : 30 solution [Fig. 2(b)]. When the S-ESM/PVA blend ratio at 60 : 40, uniform fibers were formed without beads, as shown in Figure 2(c). Further decrease in the S-ESM content, such as the ratio of S-ESM/PVA = 50 : 50 [Fig. 2(d)], resulted in fibers adhering together and an increase in the aver-

age fiber diameter. The fiber diameters measured for various samples are listed in Table II.

The S-ESM/PVA blends required higher supporting polymer content than S-ESM/PEO to produce uniform fibers. Uniform fibers were formed using a 18 wt % solute concentration with a ratio of S-ESM/PVA = 60 : 40. The results suggest that fiber formation depends on the supporting polymer and the blend ratio of S-ESM and polymer. S-ESM/PEO (95 : 5) with 20 wt % solute concentration and S-ESM/PVA (60 : 40) with 18 wt % solute concentration were used to produce uniform fiber webs for the following treatments with catechin.

#### Morphology of S-ESM blend fibers with catechin treatment

Catechin was added by immersing S-ESM/PEO (95 : 5) and S-ESM/PVA (60 : 40) as-spun fibers into catechin/ethanol solution for 1, 3, and 7 days. Ethanol was used as the solvent for catechin, due to the low solubility of S-ESM in ethanol and the insolubility of both PEO and PVA in ethanol, in addition to its nontoxicity compared with methanol. The change in the morphology of the fibers after treatment with 10 wt % catechin solution is shown in Figure 3. There was little effect of immersion time on the morphology of the S-ESM/PEO blend. Examination of the S-ESM/PVA fibers showed that a membrane-like morphology was obtained after 7 days catechin



**Figure 2** FE-SEM micrographs of (a) S-ESM/PVA = 80 : 20 as-spun fibers with 18 wt % solutes concentration, (b) S-ESM/PVA = 70 : 30 as-spun fibers with 20 wt % solutes concentration, (c) S-ESM/PVA = 60 : 40 with 18 wt % solutes concentration, and (d) S-ESM/PVA = 50 : 50 as-spun fibers with 16 wt % solutes concentration.

treatment, which suggests that the interaction between S-ESM/PVA and catechin may stronger than that for S-ESM/PEO. Accordingly, a catechin treatment duration of one day was found to be suitable to maintain the original fiber morphology. When the S-ESM/PEO (95 : 5) as-spun fibers were treated with 40 wt % catechin solution, the fibers became brittle, which indicates that treatment with a higher concentration catechin is not favorable for maintaining the fiber form. Siebert et al. reported that both protein and polyphenol molecules had a certain amount of binding sites to interact with each other.<sup>18</sup> The largest network can be produced when the number of polyphenol ends equals the number of protein binding sites. However, if there is a lack of protein relative to polyphenol, then the binding sites are not saturated, which leads to a low amount of bridging. Therefore, in the case of treatment with 40 wt % catechin solution, the binding sites of S-ESM might be fully saturated and the free catechin ends have no free binding sites on S-ESM molecules, so that the fiber form would not be maintained after treatment with catechin.

#### Insolubility of fibers in water

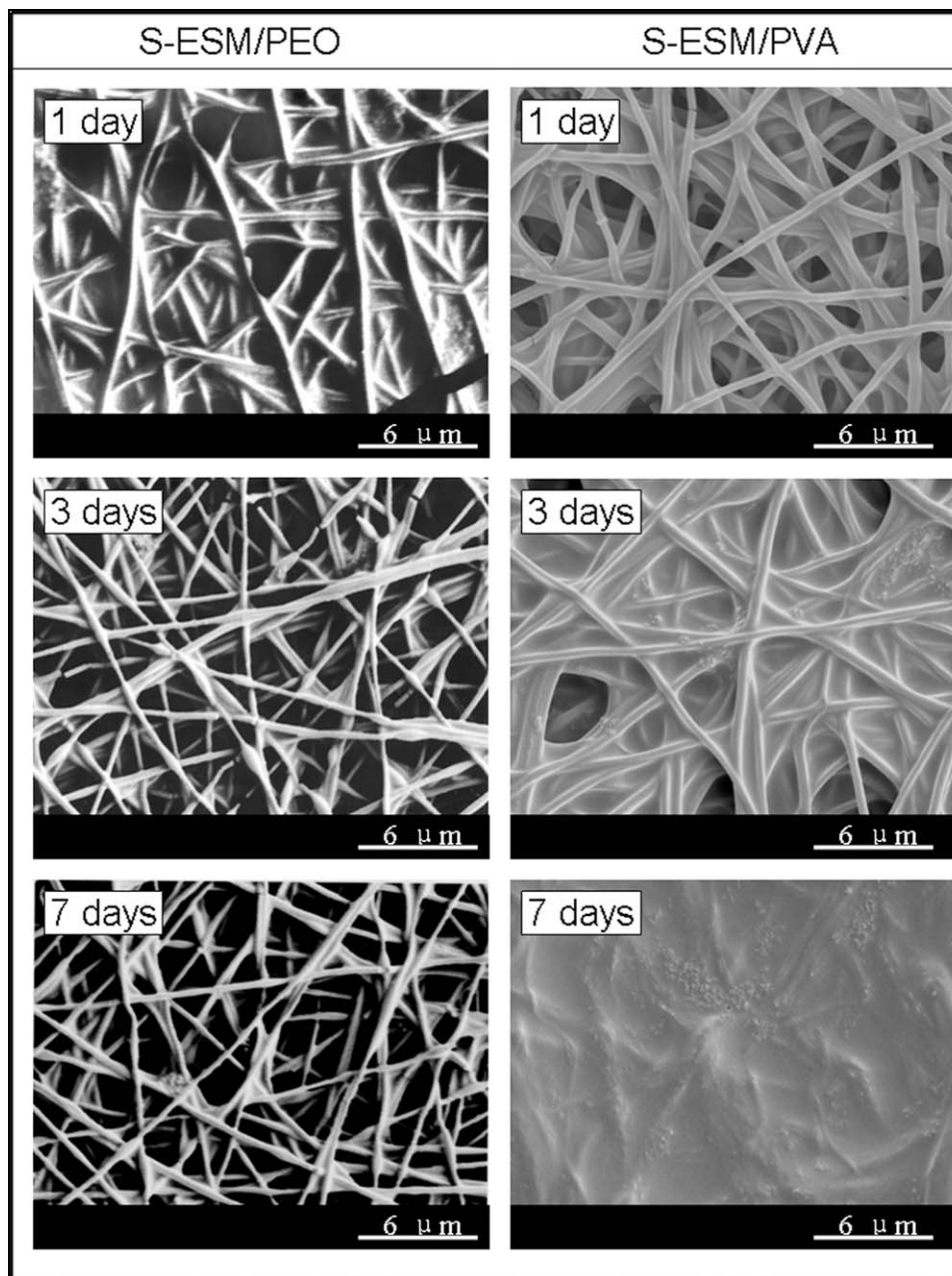
When the S-ESM/PEO (95 : 5) as-spun fibers were immersed in distilled water, they were immediately dissolved. The same phenomenon was found for

S-ESM/PVA (60 : 40) as-spun fibers in water. On the other hand, when the catechin-treated fibers were immersed in distilled water for 12 h, all samples remained insoluble. These results suggest that the insolubility of as-spun fibers is improved after catechin treatment.

In the case of the 10 wt % catechin-treated S-ESM/PEO (95 : 5) fibers, some fibers started to gradually dissolve in distilled water. However, a small amount of insoluble fiber-like material still remained after 12 h, as shown in Figure 4(a). When the 40 wt % catechin-treated S-ESM/PEO (95 : 5) sample was immersed in water, a small amount of insoluble particle aggregate was produced rather than fibers,

**TABLE II**  
The Average Diameter of As-Spun Fibers

	Blend ratio (w/w)	Solutes concentration (wt %)	Diameter (nm)
S-ESM/PEO	95 : 5	15	Beaded fiber
	95 : 5	20	240 ± 30
	90 : 10	20	262 ± 51
	80 : 20	20	235 ± 57
S-ESM/PVA	80 : 20	18	Beaded fiber
	70 : 30	20	332 ± 103
	60 : 40	18	335 ± 65
	50 : 50	16	522 ± 126



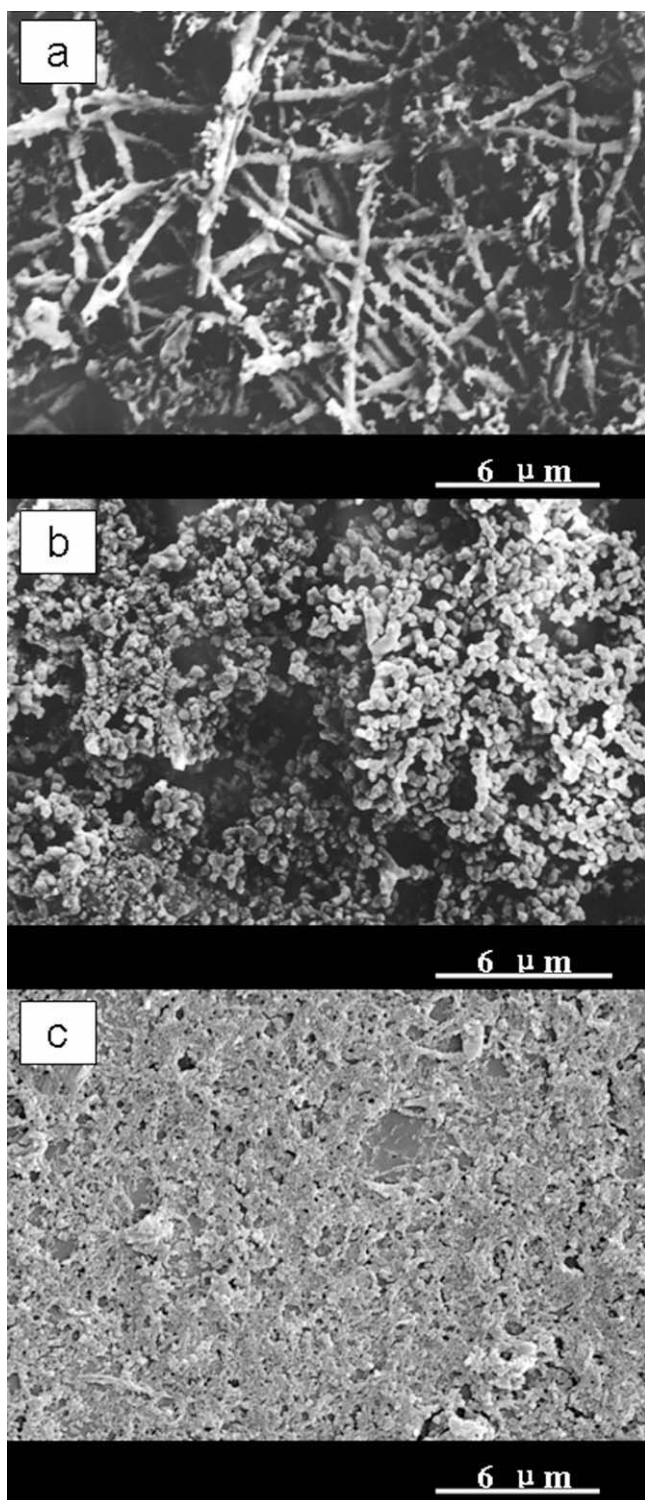
**Figure 3** FE-SEM micrographs of S-ESM/PEO = 95 : 5 and S-ESM/PVA = 60 : 40 as-spun fibers after 10 wt % catechin/ethanol treatment.

because of the high catechin to S-ESM content [Fig. 4(b)]. This result also supports that the binding sites of S-ESM might become fully saturated, so that catechin treatment results in loss of the fiber form. Thus, the 10 wt % catechin-treated S-ESM/PVA (60 : 40) fibers were dipped in water to examine improvement of the insolubility. The morphology of the insoluble material is shown in Figure 4(c). The individual fibers assumed a membrane-like shape compared with the original fiber structure. When comparing the area of insoluble material on the aluminum foil, the 10 wt % catechin-treated S-ESM/

PVA fibers covered larger area than the S-ESM/PEO fibers.

#### FTIR spectroscopy

The conformation of the S-ESM and catechin structure was determined by FTIR. The FTIR spectra of S-ESM powder, catechin powder and the precipitation of S-ESM/catechin (50 : 50, w/w) are shown in Figure 5. The FTIR spectrum of S-ESM has characteristic peaks at 1642 and 1549  $\text{cm}^{-1}$  that indicate a carbonyl band (amide I) and N—H bending (amide II),

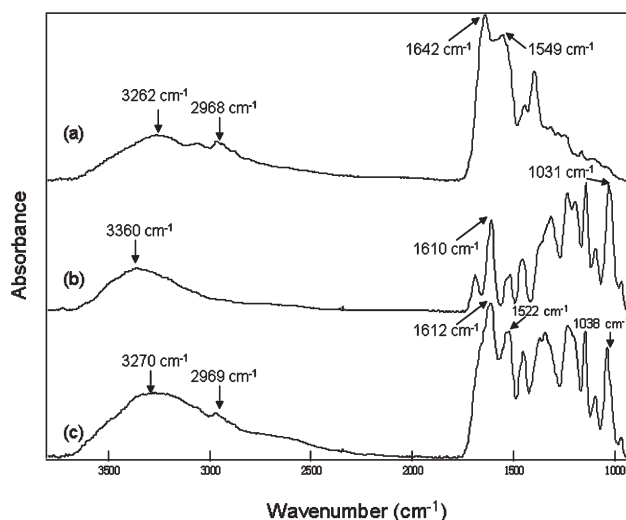


**Figure 4** FE-SEM micrographs of 1 day's catechin-treated fibers after immersing in water for 12 h (a) 10 wt % catechin-treated S-ESM/PEO = 95 : 5 as-spun fibers, (b) 40 wt % catechin-treated S-ESM/PEO = 95 : 5 as-spun fibers, and (c) 10 wt % catechin-treated S-ESM/PVA = 60 : 40 as-spun fibers.

respectively. N—H stretching vibration was observed at  $3262\text{ cm}^{-1}$  in addition to C—H stretching at  $2968\text{ cm}^{-1}$ . The characteristic peaks of catechin powder

were identified at  $3360$  and  $1031\text{ cm}^{-1}$ , which corresponded to O—H vibration and C—O absorption, respectively. C=C stretching in the aromatic rings of catechin was also observed at  $1610\text{ cm}^{-1}$ . After blending with catechin, the peaks of the amide I and amide II bands in S-ESM were shifted to  $1612$  and  $1522\text{ cm}^{-1}$ , respectively. The band at  $1612\text{ cm}^{-1}$  could be related to the hydrogen-bonded carbonyl vibration, because some of the carbonyl bond electron density would be utilized by hydrogen bonding, leading to the lower stretching frequency, which would imply the formation of intermolecular hydrogen bonds between S-ESM and catechin.<sup>20</sup> The C—O absorption at  $1038\text{ cm}^{-1}$  confirmed the presence of catechin. In the  $3200\text{--}3400\text{ cm}^{-1}$  range, the hydroxyl vibration band was decreased, whereas the N—H stretching of S-ESM was shifted to higher frequency. The relative absorbance of these two bands was observed at  $3270\text{ cm}^{-1}$ , which also supports hydrogen bond interaction. However, the combination of both N—H stretching vibration and O—H vibration in the  $3200\text{--}3400\text{ cm}^{-1}$  range means that the peak shift of this range that could confirm the formation of hydrogen bonds might not be as clear as the range of  $1500\text{--}1650\text{ cm}^{-1}$ . Madhan et al. also reported that the —C=O stretching (amide I) of collagen was shifted after catechin treatment as a result of hydrogen bond formation, whereas the absorbance at  $3300\text{ cm}^{-1}$  was only broadened.<sup>19</sup>

FTIR spectra of PEO, S-ESM/PEO, and S-ESM/PEO/catechin are shown in Figure 6. The FTIR spectrum of PEO [Fig. 6(a)] has a strong absorption peak at  $1099\text{ cm}^{-1}$ , which corresponds to the stretching of the C—O—C group.<sup>21</sup> This peak was also observed in the S-ESM/PEO as-spun fibers at  $1100\text{ cm}^{-1}$ , as shown in Figure 6(b), in addition to a shift of the amide I and amide II bands to higher frequency

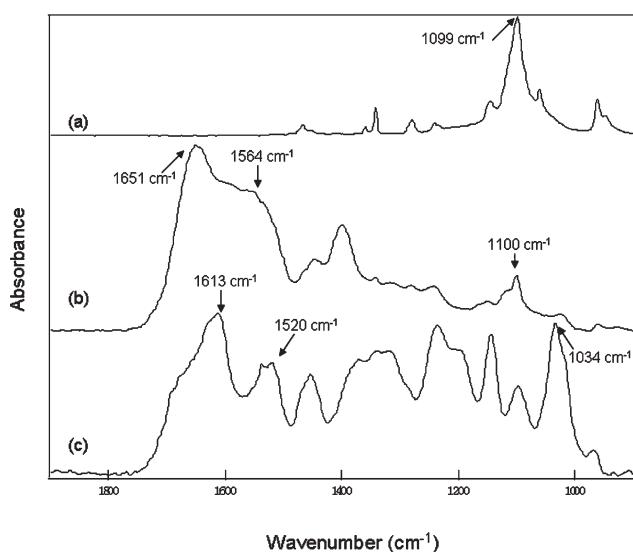


**Figure 5** FTIR spectra of (a) S-ESM, (b) catechin, and (c) S-ESM and catechin.

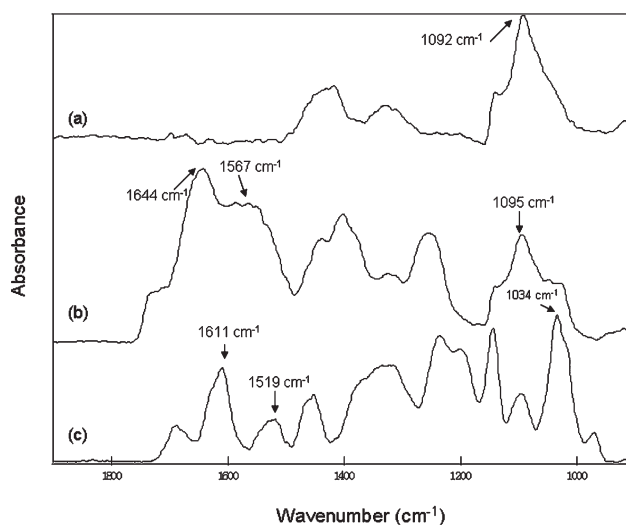
after incorporation of PEO with S-ESM. After treatment of the as-spun fibers with catechin, the characteristic peaks of the amide I and amide II bands decreased and shifted to lower frequency at 1613 and 1520  $\text{cm}^{-1}$  [Fig. 6(c)], which implies hydrogen bonding interaction as for the S-ESM/catechin precipitation. A C—O absorption band was also observed at 1034  $\text{cm}^{-1}$ , which confirmed the presence of catechin.

FTIR spectra of PVA, S-ESM/PVA, and S-ESM/PVA/catechin are shown in Figure 7. The characteristic peak of PVA was obtained at 1092  $\text{cm}^{-1}$ , due to C—O stretching and O—H bending, as shown in Figure 7(a).<sup>22</sup> Figure 7(b) shows the FTIR spectrum of S-ESM/PVA as-spun fibers. The amide I and amide II bands were observed at 1644 and 1567  $\text{cm}^{-1}$ , and these two peaks were shifted to 1611 and 1519  $\text{cm}^{-1}$  after catechin treatment, as shown in Figure 7(c). The C—O absorption of catechin was also observed at 1034  $\text{cm}^{-1}$ . The results agree well with those for the PEO blend and confirm the interaction between S-ESM and catechin. Furthermore, the large amount of —OH groups in the PVA molecular structure may also favor the formation of hydrogen bonding between S-ESM and catechin, because the characteristic peaks of the amide I and amide II bands were shifted to lower frequencies than in the S-ESM/PEO/catechin blend system. Thus, a large proportion of insoluble fiber-like structures could be obtained after treatment with catechin.

The results suggest that catechin could be used as a suitable crosslinking material for combination with S-ESM nanofibers to improve insolubility. However, the present results may not accurately reflect the amount of catechin present in the fibers, and how



**Figure 6** FTIR spectra of (a) PEO 900 K, (b) S-ESM/PEO = 95 : 5 as-spun fibers, and (c) after 10 wt % catechin-treated.



**Figure 7** FTIR spectra of (a) PVA, (b) S-ESM/PVA = 60 : 40 as-spun fibers, and (c) after 10 wt % catechin-treated.

the volume of catechin influences the insolubility of the as-spun fibers. Further experiments are required using a more effective process for the addition of catechin and control of the volume added.

## CONCLUSIONS

S-ESM nanofibers were successfully electrospun with PEO and PVA using aqueous media. Uniform fibers without beads were produced for a S-ESM/PEO blend ratio of 95 : 5 and a 20 wt % total solute concentration. A blend of S-ESM/PVA = 60 : 40 was found to be the optimum ratio for uniform fiber formation. The results suggest that catechin plays an important role in improving the insolubility of both S-ESM/PEO and S-ESM/PVA blend as-spun fibers in water through the hydrogen bond interactions between S-ESM and catechin. The insolubility of electrospun fibers was improved by immersing the as-spun fibers in a 10 wt % catechin/ethanol solution. Changes in morphology were observed at each stage of processing; after catechin/ethanol treatment and with immersion in water. A larger proportion of insoluble fiber-like structure material remained for S-ESM/PVA/catechin webs compared to S-ESM/PEO/catechin fibers after immersing in water. FTIR spectroscopy confirmed the interaction between S-ESM and catechin, and also suggested a stronger interaction between S-ESM/PVA as-spun fibers and catechin than S-ESM/PEO and catechin. Further research is required to develop more effective processes for the addition of catechin, and to determine how the volume of catechin influences the improvement of insolubility. The S-ESM and catechin fibers obtained in this study could be potentially applied as wound dressings or as cosmetic sheets in the biomedical field.

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