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Preparation and Characterization of Electrospun Polycaprolactone Nanofiber Webs Containing Water-soluble Eggshell Membrane and Catechin

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Abstract

The process of incorporating water-soluble Eggshell Membrane (S-ESM) into Polycaprolactone (PCL) electrospun nanofiber webs was investigated, utilizing the hydrogen bonding interaction between S-ESM and catechin. PCL was first electrospun with catechin, and S-ESM was introduced into the resulting PCL/catechin nanofibers by immersing the fibers in S-ESM solution. Morphological observation suggested that S-ESM was incorporated with catechin and formed S-ESM/catechin nanoparticles, distributed in the PCL nanofiber webs. Analysis of FTIR spectra indicated that hydrogen bonding interactions were generated between PCL and catechin as well as between S-ESM and catechin. The bulk compression property of electrospun nanofiber webs was measured and the results showed S-ESM and catechin had effect on modifying the mechanical property of PCL nanofiber webs.

Keywords: Water-soluble Eggshell Membrane; Catechin; Nanofiber; Compression

1 Introduction

In recent years, the electrospun nanofibers have attracted much attention due to the nano-scale diameter, high surface area to volume ratio, and unique physical properties, which make them suitable to widely potential applications. The nanofibers fabricated by using natural compounds have been investigated as good candidate for medical materials [1-2]. However, the electrospinning of natural materials is limited by their complex chemical contents which lead to difficulty in preparing efficient spinning dope. Furthermore, to achieve medical application of natural material nanofibers, the mechanical properties of nanofibers needs to be studied. Nevertheless, the characterization method for understanding the mechanical properties of nanofiber webs is not yet satisfied, since nanofiber webs are naturally soft, nano-scale in diameter, and few micrometers in thickness. The effective characterization method is required to evaluate the electrospun nanofiber webs.

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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 [3]. The natural insolubility of ESM is a main obstacle to further application, and in particular, the production of ESM fibers. Rather than employing natural ESM, water-soluble ESM (S-ESM) from natural hen ESM has been considered. Nevertheless, the electrospinning of aqueous S-ESM is difficult due to the low viscosity. In our previous study, electrospinning of S-ESM was carried out by blending S-ESM with poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA) for the S-ESM fiber formation [4]. To maintain the fibrous structure of the soluble as-spun S-ESM/PEO and S-ESM/PVA fibers in water, the effect of incorporation of catechin with the S-ESM/PEO and S-ESM/PVA fibers was studied. Catechin is a non-toxic, polyphenolic antioxidant plant metabolite that belongs to the flavonoid family, and that has been shown to possess antibacterial, antioxidant, and other physiological functions. Furthermore, many studies have reported that the hydrogen bonding and hydrophobic interactions can be generated between protein and polyphenol [5-6], therefore, the interaction between S-ESM and catechin was examined to maintain the fibrous structure of S-ESM/PEO and S-ESM/PVA as-spun fibers in water. However, the result indicates only a few S-ESM/catechin precipitate with fibrous structures were observed because some of the S-ESM failed to interact with catechin and was probably dissolved in water with PEO or PVA. Moreover, the obtained S-ESM/catechin precipitate was also brittle in nature which leads to inefficient mechanical property. 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 aliphatic polyester that has been widely considered as a candidate material for medical application, due to its excellent mechanical properties, biodegradability, and biocompatibility. Several processes have been reported for incorporating S-ESM with aliphatic polymers. In one of these processes, Lu et al. reported the process to entrap the Soluble Eggshell Membrane Protein (SEP) onto poly(D, L-lactic acid) PD-LLA membrane [7]. In order to explore an effective and simple process to add S-ESM into electrospun PCL nanofibers, catechin was considered to be used. Zhu et al. reported that an intermolecular hydrogen bonding interaction was generated between the ester carbonyl groups of the polyesters, and the phenol groups of catechin [8]. 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 the present study, we aim to investigate using catechin as a key material for the fabrication of PCL-based S-ESM/catechin nanofiber webs, and investigate the effect of addition catechin and S-ESM on modifying the mechanical properties of PCL nanofiber webs. First, a process to prepare electrospun PCL nanofibers containing S-ESM and catechin was developed and analyzed. Secondly, the bulk compression property of electrospun nanofiber webs was measured using a modified compression tester (Kawabata evaluation system, KES-G5s) for understanding the fundamental mechanical property of PCL-based S-ESM/catechin nanofiber webs. The PCL-based S-ESM/catechin nanofibers could combine all the benefit properties of materials and have potential to be in medical application, such as wound dressing.

2 Experimental

2.1 Materials

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

2.2 Preparation and Electrospinning of PCL/catechin Spinning Dopes

The PCL pellets were dissolved in CH_2Cl_2/DMF co-solvents (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 1. The dynamic viscosity of PCL and PCL/catechin dopes were measured by viscosimeter (model: RVDV-II +P CP, Brookfield, USA) at 25 ℃. 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 20cm. 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 1.

Table 1: 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 ℃, 40% (R.H)

2.3 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 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) co-solvent 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, since 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.

2.4 FTIR Analysis

Attenuated Total Reflection (ATR) Fourier transform infrared 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.

2.5 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 mean diameter of individual electrospun nanofibers was evaluated using Image J listed in Table 2.

Table 2: The mean fiber diameter

PCL:catechin (w/w)	Diameter (nm)
100:0	283±98
95:5	407±138
90:10	491±202
85:15	549±293
80:20	762±221

2.6 Compression Test

For the sample preparation of compression test, the spinning dopes were electrospun directly onto an aluminum stage, according to our previous experiment [9]. Random fibers were formed and dried in a desiccator for 24hrs then transferred to the KES-G5s compression tester (Kato Tech. Co., Ltd.). Spinning was carried out approximately for 3hrs for all spinning dopes. The approximate thickness is increased with increasing catechin content and in the range of 30-80 μm. The S-ESM treated sample was obtained by immersing the PCL/catechin as-spun fibers with aluminum stage in S-ESM solution and then vacuum dried under flat state in a desiccator at room temperature following the same process mentioned in the previous section.

The compression measurement was carried out with a ball type indenter (ϕ=5 mm) under a compression speed of 1μm/s and maximum forces of 1.0N in room condition as shown in Fig. 1. Five different positions were selected from the surface of a nanofiber webs sample, and each position was compressed for 3 times. The Maximum Displacement (MD) at maximum compression force 1.0N, compression energy to reach the maximum load (WC-N) and Resilience (RC) were calculated from the load-displacement curve.

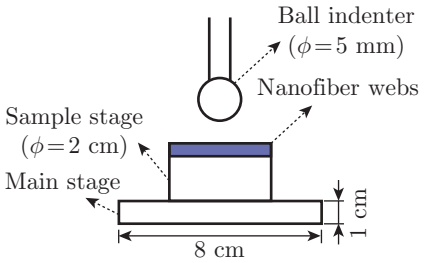

Fig. 1: Schematic diagram of compression measurement setup

3 Results and Discussion

3.1 FTIR Spectroscopy

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. 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 [3].

FTIR spectra of pure PCL and PCL/catechin as-spun fibers at catechin blend ratios of 5 wt% to 30 wt% are shown in Fig. 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. also observed that the relative absorbance of hydrogen-bonded carbonyl vibration increased with increasing catechin content of polyester/catechin blend films [6]. 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.

The FTIR spectra of pure PCL and PCL/catechin as-spun fibers after S-ESM treatment are shown in Fig. 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, 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 previous results and the FTIR results in Fig. 2.

3.2 Morphology

The effect of catechin weight ratios of blended dopes on fiber morphology was investigated. The morphology of PCL/catechin blend fibers is shown in Fig. 4. Fine uniform blend fibers were electrospun at blend ratios of PCL:catechin of 95:5 and 90:10 (Fig. 4 (b) and 4 (c)). When the

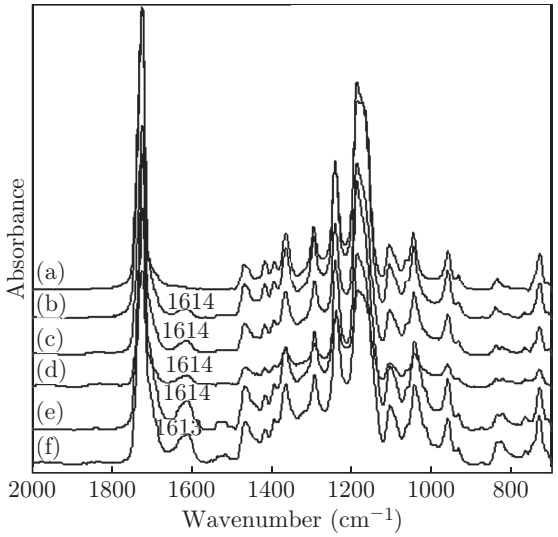

Fig. 2: FTIR spectra of as-spun fibers (a) pure PCL, (b) PCL: catechin = 95:5, (c) PCL: catechin = 90:10, (d) PCL: catechin = 85:15, (e) PCL: catechin = 80:20, and (f) PCL: catechin = 75:25

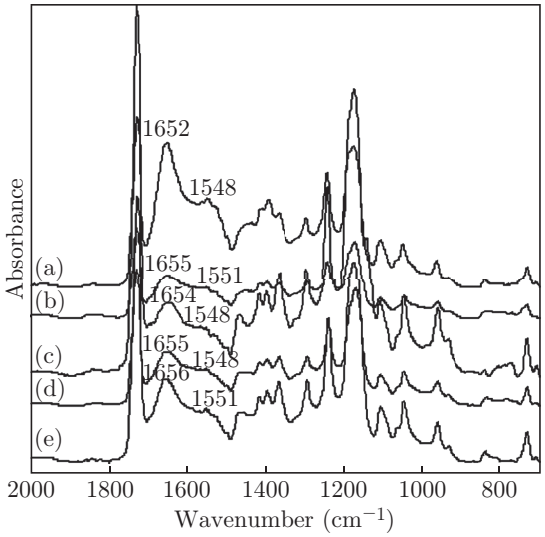

Fig. 3: FTIR spectra of S-ESM treated as-spun fibers made from (a) pure PCL, (b) PCL: catechin = 95:5, (c) PCL: catechin = 90:10, (d) PCL: catechin = 85:15, and (e) PCL: catechin = 80:20

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)) compared to fibers prepared using a low catechin content (Fig. 4 (b) and 4 (c)). When solutions composed of PCL/catechin at ratios of 75:25 was electrospun, solution droplets and only a few fibers were observed on the collector. An increase in the mean fiber diameter was observed with increasing catechin content (Table 2). The relationship between viscosity and catechin content of spinning solutions was shown in Fig. 5. The viscosity of solution was increased with increasing 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

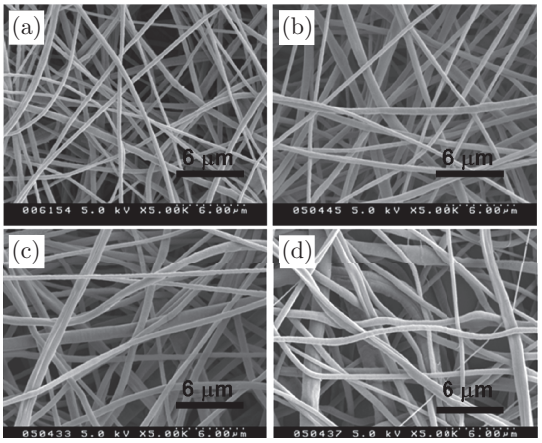

Fig. 4: FE-SEM micrographs of as-spun fibers made from (a) pure PCL, (b) PCL: catechin = 95:5, (c) PCL: catechin = 90:10, (d) PCL: catechin = 85:15

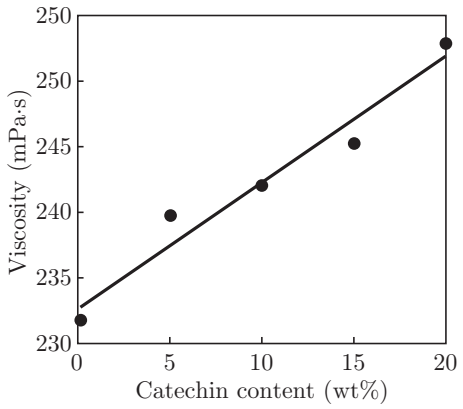

Fig. 5: The effect of catechin content on the viscosity of PCL/catechin solutions

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.

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 Fig. 6. A membrane-like morphology was observed for the S-ESM treated PCL and PCL:catechin=95:5 nanofibers (Fig. 6 (a) and 6 (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. 6 (c) and 6 (d)). In Fig. 7, 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.

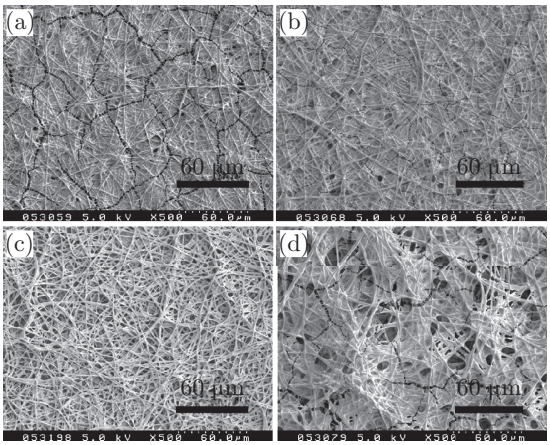

Fig. 6: Low magnification. FE-SEM micrographs of S-ESM treated fibers: (a) PCL: catechin = 95:5, (b) PCL: catechin = 90:10, (c) PCL: catechin = 85:15, and (d) PCL: catechin = 80:20

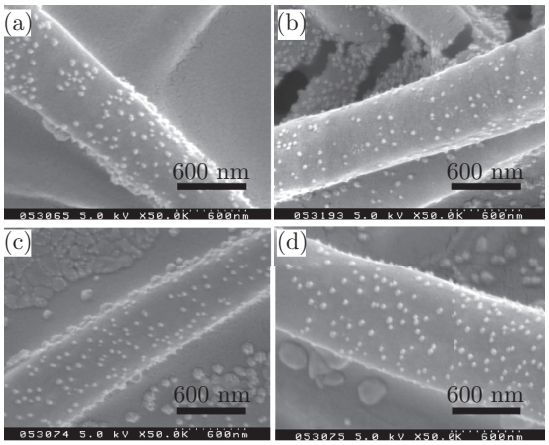

Fig. 7: High magnification. FE-SEM micrographs of S-ESM treated fibers: (a) PCL: catechin = 95:5, (b) PCL: catechin = 90:10, (c) PCL: catechin = 85:15, and (d) PCL: catechin = 80:20

The results of FTIR spectra and morphology observation show that fundamentally, 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.

3.3 Compression Test

Fig. 8 shows load-displacement curves (five specimens) of PCL nanofiber webs after subtracting the displacement of aluminum stage. The result suggests the smooth typical compression curves

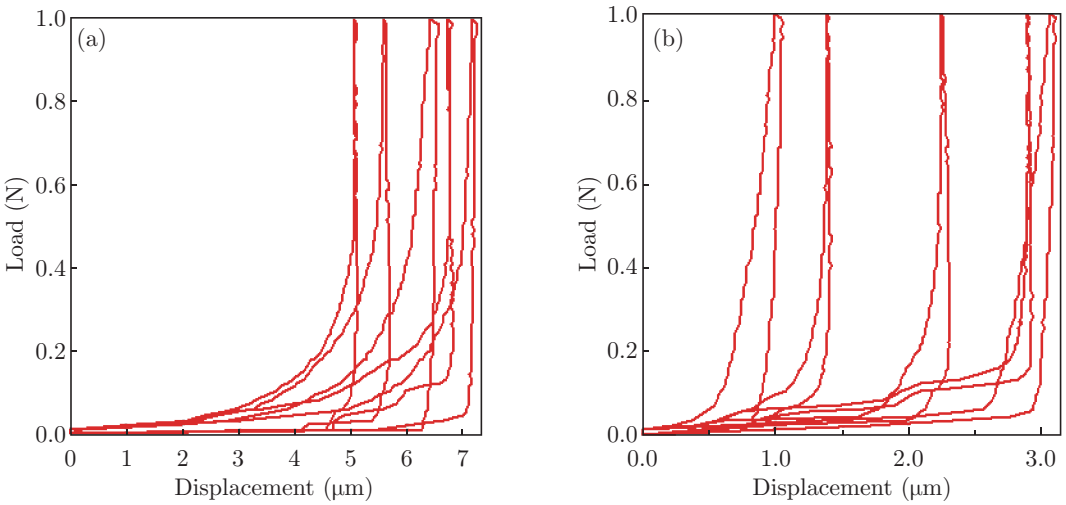

Fig. 8: Load-displacement curves for electrospun PCL nanofiber webs (a) 1 st compression and (b) second compression

Table 3: Compressional parameters of PCL/catechin electrospun nanofibers

PCL/catechin (w/w)		MD (μm) 1 st curve	MD (μm) 2 nd curve	WC-N (μJ) 1 st curve	WC-N (μJ) 2 nd curve	RC (%) 2 nd curve
100:0	Average	6.2	2.1	0.59	0.19	37.01
	SD	0.9	0.9	0.10	0.06	17.16
95:5	Average	17.1	7.4	2.11	0.75	55.86
	SD	3.8	4.1	0.42	0.20	17.33
90:10	Average	22.6	3.6	3.72	0.90	32.72
	SD	5.1	0.9	0.92	0.29	8.46
85:15	Average	44.2	13.2	6.30	2.00	27.83
	SD	3.8	3.0	0.57	0.19	1.57
80:20	Average	49.7	13.7	6.51	2.07	29.38
	SD	5.1	2.9	0.51	0.30	4.21

SD: Standard Deviation

were obtained for Electrospun PCL nanofiber webs. The MD, WC-N, and RC were calculated from the curves of PCL nanofiber webs with different catechin ratios and listed in Table 3. The result indicates that the average MD and WC-N calculated from the first compression was higher than the one from the second compression at all PCL/catechin blend ratios. It is possible the layer-like porous structure of electrospun nanofiber webs exist in the space between each layers resulting in higher value of MD and WC obtained in the first compression. When the nanofiber webs were compressed, the nanofibers moved around the ball indenter and density was changed under the applied force, and the space between each layer was lost due to the adhesion between each layer. In the second compression, the movement between fibers is reduced due to the adhesion caused by the first compression. Therefore, the MD and WC-N calculated from the second compression shows smaller value than that of the first compression. It is necessary to point out that since the first compression curve was mainly affected by the movement of the fibers, the large hysteresis

observed from the first compression curve can be attributed to the interfiber friction. Therefore, the RC calculated from the second compression curve was more reliable to express the compression resilience of the PCL nanofiber webs.

Furthermore, the MD and WC-N, in both of first and second compression, were increased with the increasing of catechin content in the PCL/catechin nanofiber webs. This is because the nanofiber webs produced by higher catechin content solution consist of larger fiber diameter resulting in higher thickness of nanofiber webs than the low catechin content one. The RC of PCL/catechin was almost equivalent with pure PCL except the 5 wt% catechin content sample. The effect of catechin content on RC values was not clearly observed, probably fiber friction between fibers dominate the compression resilience of nanofiber webs.

When PCL/catechin nanofiber webs were treated by S-ESM, the MD and WC-N calculated from the first compression and second compression were also increased with the increasing of catechin content. The MD and WC-N values of S-ESM treated PCL/catechin samples are smaller than those of untreated samples as shown in Fig. 9. Adding S-ESM might generate the interaction to catechin which may cause webs to become stiffer. The RC value of PCL/catechin with S-ESM treatment was small with the increase of catechin content. Since the S-ESM membrane was formed between the PCL/catechin fibers, this structure might have effect on the changes in RC. The compression measurement results suggest the modified KES-G5s tester could apply to the evaluation of nanofiber webs, and the addition of catechin and S-ESM has effect on modifying the mechanical property of PCL nanofiber webs.

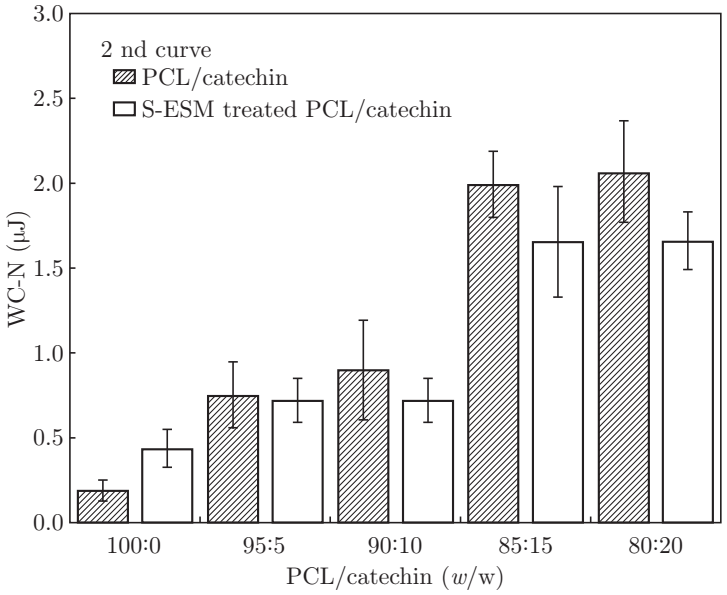

Fig. 9: The WC-N of PCL/catechin and S-ESM treated PCL/catechin nanofiber webs

4 Conclusion

In the present study, the process of incorporating water-soluble eggshell membrane (S-ESM) into polycaprolactone (PCL) electrospun nanofibers was examined to determine its effect on the generation of PCL-based S-ESM/catechin nanofibers. The non-toxic 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 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 which can be observed 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 the S-ESM treatment of pure PCL nanofibers. The bulk compression property of electrospun nanofiber webs was measured by modified KES-G5s compression tester. The results indicated that the MD and WC-N calculated from the second compression shows smaller value than that of the first compression for all the samples due to the fiber movement and adhesion caused by the first compression. It is also found the addition of catechin and S-ESM had effect on modifying the mechanical property of PCL nanofiber webs. Further experiments are required to improve the immersion process for the addition of S-ESM into PCL/catechin as-spun fibers in order to form PCL-based S-ESM/catechin nanofibers. The more details of the compression property of nanofiber webs need to be understood. The PCL-based nanofibers with S-ESM/catechin nanoparticles could have potential application as wound dressing in medical field.

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