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Characterization of gelatin nanofiber prepared from gelatin–formic acid solution

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Abstract

Gelatin, well known as a biocompatible polymer, was dissolved in formic acid and gelatin nanofiber was successfully prepared by the electrospinning using gelatin–formic acid dope solution. Stability of the dope solution was evaluated by measuring viscosity change with time. Even though the viscosity dropped markedly after 5 h, the spinnability and morphology of gelatin nanofiber were not affected at all. The parameters, such as electric field, spinning distance, and concentration of dope solution, were examined for studying the effects on electrospinnability and morphology (size, size distribution, uniformity, bead formation, etc.) of gelatin nanofiber web. The gelatin nanofibers, in the mean size of 70–170 nm, could be prepared by controlling the dope concentration under proper conditions. The electrospun gelatin nanofiber exhibited a mixture of α-helical and random coil conformation, which was amorphous structure with very low crystallinity. The structural transformation, from a helical (α-helix and triple-helix) to random coil conformation, might occur when formic acid was used for the dissolution of gelatin in electrospinning.

Keywords: Electrospinning; Gelatin; Nanofiber

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1. Introduction

Electrospinning, which was introduced in early 1930s, is known as a novel fabrication method for producing ultra fine fiber in a nano-scale with simple apparatus. Ultra fine fibers of various sizes (from few tens nanometers to micrometers), which can have large specific areas, are formed under a high electric field by this method. Electrospinning technique can apply to not only synthetic polymers but also natural polymers. Many studies have been reported for the electrospun polyester, PVA, PEO, nylon, collagen, and silk fibroin [1–7]. Recently, the electrospun nanofibers would be considered in biomaterials as a high performance filter, wound-dressing, drug delivery system, and cell-growth scaffold due to its high surface to volume ratio [8–13].

Particularly, since bioengineering field received much interest, the electrospinning of natural polymers has been studied in recent time. However, although the natural polymers have good properties for biomedical engineering application field, the electrospinnable natural polymers are limited because of the difficulty of handling. Among the natural polymers, gelatin, which can be obtained by denaturing collagen, is very conventional one. Gelatin is known to have biocompatibility and biodegradability similar to those of collagen. Furthermore, it can be easily obtained by extracting from animal tissue such as skin, muscle, and bone. For that reason, there are many studies to fabricate the gelatin to various forms for wide applications [15–19]. However, there was few attempts to form nanofibrous gelatin by electrospinning [20].

In this study, we tried to develop the novel dope solution of gelatin for electrospinning and to produce gelatin nanofibers. Generally, gelatin can be dissolved in warm water and fabricated in various forms. But it was impossible to obtain gelatin nanofiber by electrospinning because the aqueous gelatin solution easily turned to gel in the syringe needle at room temperature. As well, water was not

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appropriate for electrospinning of gelatin because the solution must be quickly volatilized to solidify the electrospun thread. Therefore, formic acid, known as a very volatile solvent, was employed as a solvent for dissolution of gelatin. Furthermore, it is another advantage to dissolve gelatin in this solvent at room temperature without gelation. These properties made it possible to fabricate the electrospun gelatin nanofibers. However, the degradation of gelatin molecules in formic acid can be occurred slightly due to its high acidity. Thus, the stability of gelatin–formic acid solution was examined.

For the preparation of gelatin nanofibers, various factors, which affects on the electrospinning process, were studied in detail. And the electrospun gelatin nanofibers prepared from formic acid solution were characterized using FTIR spectroscopy, Circular dichroism, XRD, and DSC in order to verify the conformation and crystallinity.

2. Experimentals

2.1. Materials

Gelatin (from porcine skin, Sigma Aldrich) and formic acid (98%, Kanto Ltd) were used for dope solution without any treatment or further purification.

2.2. Film casting

Gelatin solution of 1.5 wt% concentration was prepared by dissolving the gelatin in distilled water and 98% formic acid. Gelatin film was also prepared by pouring each solution into petri-dish and evaporating the solvent.

2.3. Electrospinning

Gelatin was dissolved in formic acid with various concentrations (7–12 wt%) and the solution was stirred for 1 h at room temperature. To remove insoluble fraction or impurities, the solution was filtered with non-woven fabric. For electrospinning, each dope solution was placed in a syringe (10 ml) with stainless steel syringe needle as an electrode. The syringe was loaded in a syringe pump (KD Scientific, USA) to control a flow rate accurately. The syringe needle was connected to a power supply (Chungpa EMT high voltage supply, Korea). Rolling stainless drum was used as a collector and grounded. The flow rate of dope solution was controlled to maintain a constant size of droplet at the end of the syringe needle. The electrospinning was performed at room temperature and below RH 60%. To evaluate the effect of dope stability on the spinnability, the electrospinning was performed on the specimens with different storage time of the dope.

Electric potential was controlled between 6 and 25 kV and distance to collector was between 7.5 and 25 cm for proper electrospinning conditions. At a critical voltage, the shape of droplet transforms to a cone, so-called ‘Taylor cone’ by electric force. Then, a charged solution jet was driven to the opposite charged collector and non-woven mat type of gelatin nanofiber was formed. Finally, it was dried overnight in 40 °C oven to remove moisture before measurements.

2.4. Dope solution stability

The stability of gelatin–formic acid dope solution was evaluated by measuring the viscosity with storage time. The viscosity change can be related to the decrease of molecular weight, which indicates the degradation occurs. Gelatin solutions (8, 10, and 12 wt%) were prepared by dissolving in formic acid and distilled water, respectively. The viscosity of the solution was measured at 25 and 60 °C for 48 h using viscometer (Brookfield, USA).

2.5. Characterization

Morphology of the electrospun gelatin nanofiber was examined by scanning electron microscopy (SEM) (JSM-5410LV, JEOL, Japan) and the obtained images were analyzed by image analyzing software (ImagePro, USA). Chemical structure and conformation of gelatin powder, film, and electrospun non-woven web were analyzed by FTIR (MIDAC, USA). Circular dichroism (CD) spectra of gelatin were obtained by re-dissolving each specimen in distilled water to investigate the conformation of gelatin using a circular dichroism detector (JASCO, J-715, Japan).

Furthermore, to investigate the crystallinity of the electrospun gelatin nanofiber, X-ray diffraction (XRD) analysis was performed on wide-angle X-ray diffraction with General Area Detector Diffraction System (GADDS, Bruker-Axs, Germany) using Cu Kα radiation. Irradiation conditions were 40 kV and 40 mA. X-ray diffractogram was obtained by 2θ scanning for fiber diagram. Thermal properties of the electrospun gelatin were analyzed by DSC (Thermal Analysis Instrument TA 2910). The measurement conditions were a scanning rate of 20 °C/min and nitrogen gas flow rate of 50 ml/min.

3. Results and discussion

3.1. Solution stability

Gelatin is very easy to process because of its dissolving property in warm water. But the aqueous gelatin solution changes to a gel form in a syringe of electrospinning apparatus due to temperature effect. Furthermore, water cannot be volatilized as quickly as enough to coagulate the gelatin solution, resulted in making impossible to electrospinng. Therefore, we tried to use formic acid as a novel solvent to overcome above drawbacks. Gelatin could be dissolved in formic acid, a volatile organic solvent, as much as possible at room temperature. Similar to dry spinning, volatility of the solvent would be a very important factor for the electrospinning. However, hydrolysis might occur moderately on molecular chains of gelatin in solution due to a strong acidity of formic acid. Therefore, the stability of gelatin solution in formic acid was examined by means of measuring viscosity.

The viscosity of polymer solution usually depends on the molecular weight of polymer. Accordingly, we measured the viscosity of gelatin–formic acid solution with storage times as an indirect method for measuring the molecular weight of gelatin. Fig. 1 shows the viscosity change of gelatin solution with storage time. The viscosity of aqueous gelatin solution was little changed for 48 h even at 60 °C, reflecting that the solution was very stable and the degradation did not occur at all. On the contrary, the viscosity of gelatin–formic acid solution moderately decreased with time. The viscosity of gelatin solution became a half after 48 h, indicating that the degradation occurred in formic acid for prolonged times due to a cleavage of molecular chains. As well, this was more significant and rapid at a relatively high concentration of gelatin–formic acid solution. Nevertheless, as shown in Fig. 1, the viscosity hardly decreased during the first 2–3 h of dissolving time. The viscosity measurement indicated that gelatin–formic acid solution was stable at least for 5 h at 25 °C in the concentration range of 8–12%. Even though the viscosity markedly and continuously decreased with storage time, there were no difficulties in electrospinning process and spinnability. Therefore, the gelatin–formic acid solution can be used as a dope for electrospun nanofibers.

3.2. Electrospinning

3.2.1. *Effect of storage time of dope solution*

Generally, unstable dope solution does not maintain a good spinnability during the spinning process. In the former section, it was observed that the gelatin dope solution was unstable after prolonged storage time due to hydrolysis of gelatin molecules in formic acid. In order to find out the effect of dope storage time on electrospinning, the electrospun nanofibers with different storage time were examined. Fig. 2(a) and (b) show SEM photographs of the electrospun gelatin nanofibers for dope storage time of 0 and 24 h, respectively. As unexpected, little difference of fiber morphology was observed on micrographs. Moreover, mean size of fiber diameter was also little affected by the storage time of 0–24 h (Fig. 3). Therefore, excellent spinnability can be maintained during electrospinning process, and morphology of gelatin nanofiber is not altered in spite of hydrolysis and degradation of gelatin molecules. The viscosity as well as molecular weight strongly affects the fiber size and spinnability in general. However, this is not the case in electrospun gelatin nanofibers.

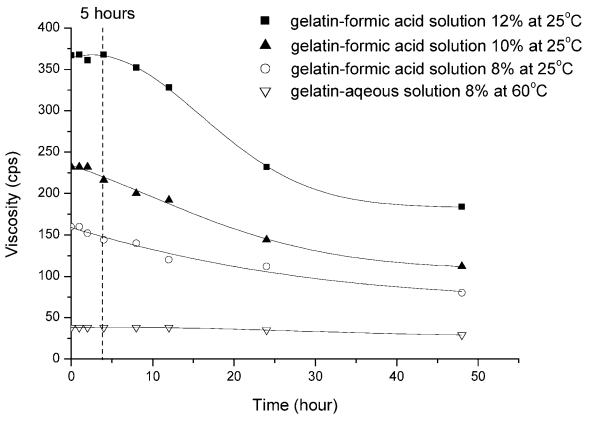
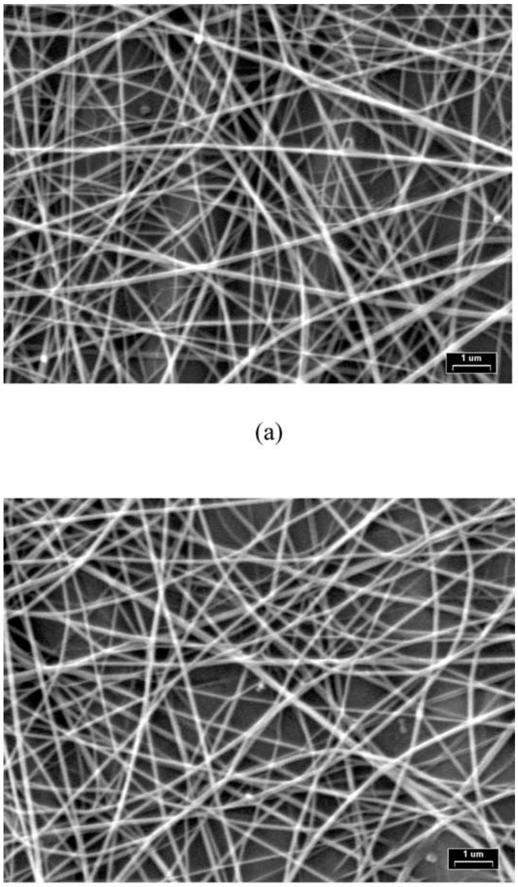


Fig. 1. Viscosity change of gelatin–formic acid solution with storage time.

3.2.2. *Effect of concentration*

For evaluating the effect of the dope concentration, the electrospinning was performed with various concentrations of 7–12 wt% under the electric field of 1.0 kV/cm and spinning distance of 10 cm. The morphological structures of electrospun gelatin nanofibers were shown in Fig. 4. SEM photographs showed that the gelatin nanofibers deposited randomly and their diameters were from several tens to a



(a)

(b)

Fig. 2. Scanning electron microscope photographs of electrospun gelatin nanofiber with dope storage time; (a) 0 h and (b) 24 h.

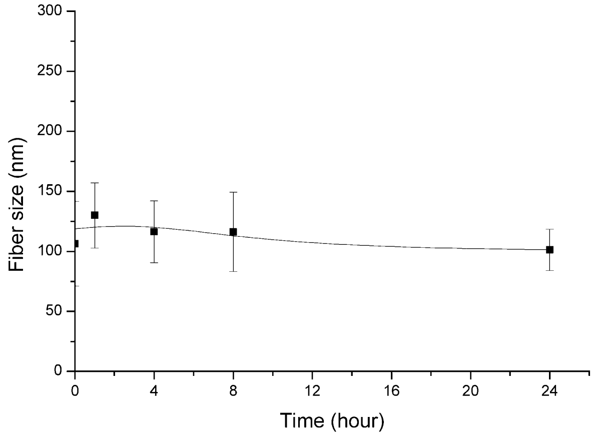


Fig. 3. Mean size of electrospun gelatin nanofiber with various dope storage times: dope concentration, electric field, and spinning distance were 8 wt%, 10 kV/cm, and 10 cm, respectively.

few hundred nanometers. Additionally, nanofibers intersected each other to make up numerous pores, whose sizes were commonly several micrometers. This can make the electrospun gelatin web having a very porous structure.

At a lower concentration (below 7 wt%), the electrospun nanofibers could not be formed just by spraying on a collector. Even though the gelatin nanofibers, which had a diameter of approximately 70 nm, were obtained at 7 wt% concentration, many beads as well as droplets were formed. This phenomenon is well known being related to the viscosity, which is varied with the concentration of the solution. Fong et al. described this as the effect of the viscosity and surface tension [14]. At a low concentration, the viscosity of the solution is low, while the surface tension is relatively high. Therefore, the solution jet, which would form a nanofiber, could not maintain its own shape at the end of tip due to high surface tension and formed a small drop among the fibers. Besides, the solution was even sprayed because of a low viscosity. These effects cause the appearance of the beads instead of the formation of fiber and this reduces a uniformity of electrospun gelatin web.

Fig. 5 showed the mean size of gelatin nanofiber ranged from 70 to 170 nm, depending on the concentration of 7–12 wt%. The fiber diameter and its standard deviation of gelatin corresponding to each concentration solution were listed in Table 1. The diameter of electrospun fiber increased as the concentration of the gelatin solution increased with a power law relationship. It also showed that the diameter increased with the viscosity of gelatin–formic acid solution due to direct proportionality between the concentration and viscosity. This has been well defined and understood in former researches [1–7,14]. The solution viscosity has been the most important parameter, which can affect the morphology of electrospun nanofiber. In case of electrospun gelatin nanofiber, a very uniform and finest nanofiber (mean diameter of about 80 nm) could be obtained at 8 wt% concentration under electric field of

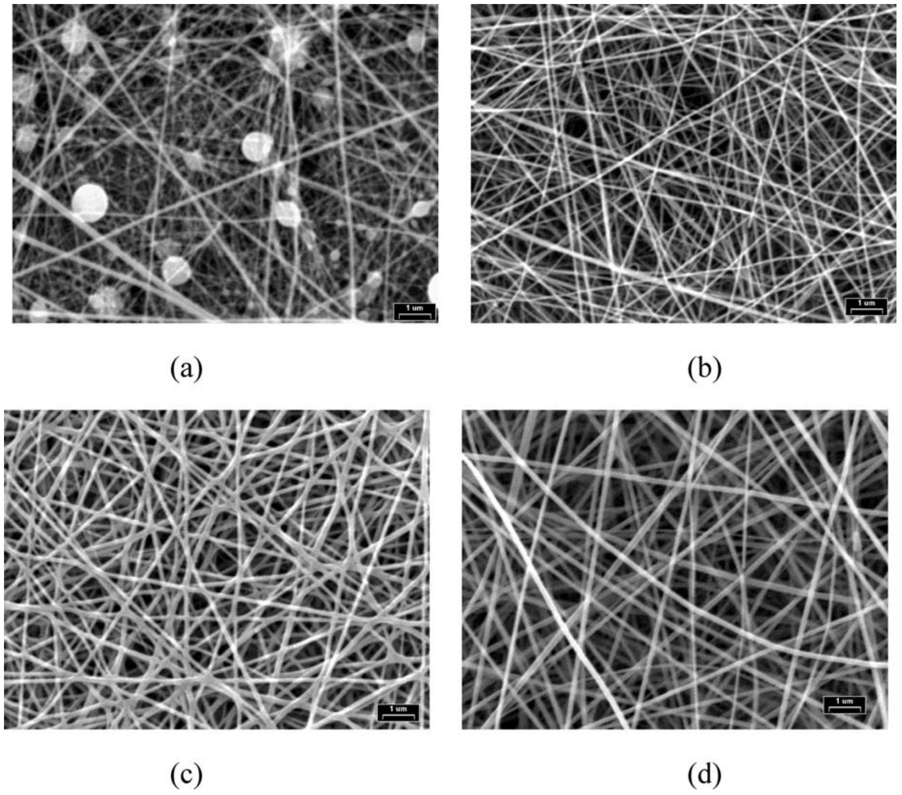


Fig. 4. Scanning electron microscope photographs of electrospun gelatin nanofibers with various dope concentrations (magnification $\times 10^4$); (a) 7%, (b) 8%, (c) 10% and (d) 12%.

Table 1
Fiber diameter variations of gelatin corresponding to the concentration solution

Concentration (wt%)	Fiber size	
	Ave. diameter (nm)	Standard deviation (nm)
7	74	16
8	76	14
9	90	19
10	131	13
11	145	19
12	169	33

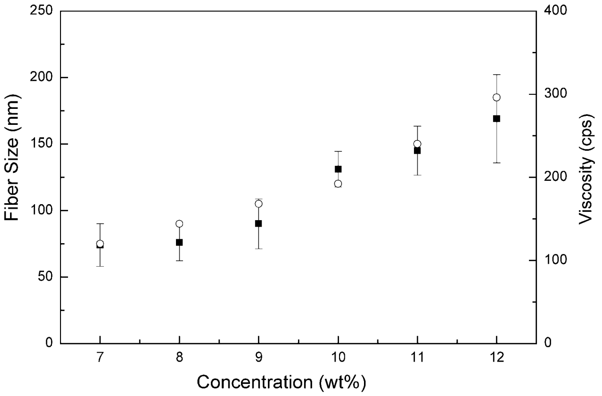


Fig. 5. Average sizes of electrospun gelatin nanofibers with various dope concentrations (solid square) which are directly related to the viscosities of dope solutions (open circle).

1.0 kV/cm and spinning distance of 10 cm. Therefore, 8 wt% concentration of dope solution was fixed throughout further experiments.

3.2.3. Effect of electric field and spinning distance

In order to examine the effect of electric field on morphology, five series of electric field were used for the study at a fixed spinning distance of 10 cm: 0.75, 1.0, 1.5, 2.0, and 2.5 kV/cm. The electrospinnability and fiber diameter of gelatin on electric field and spinning distance were listed on Table 2. It was found that 0.75 kV/cm was the critical electric field for stable electrospinning at 10 cm distance in our experiments.

Fig. 6(a) and (b) represents the morphology of gelatin nanofibers under the electric field of 0.75 and 2.0 kV/cm, respectively. Higher electric field (above 2.0 kV/cm) produced beads and droplets more frequently because spray of droplets, which were not enough charged to form a stable jet, occurred due to very high electric field. Beads or droplets are commonly expected to harm the uniformity of electrospun nanofiber web.

As a result of measuring the size and its distribution of gelatin nanofiber (Fig. 7 and Table 2), the electric field did not significantly affect the size of nanofiber. But its distribution was slightly broadened under a high electric field.

Spinning distance, which is the distance between spinneret (tip of the syringe needle) and collector (rotating drum), can be also considered as a factor for electrospinning process. Four series of the spinning distance were examined under a fixed electric field of 1.0 kV/cm: 7.5, 10.0, 15.0 and 20.0 cm. As shown in Fig. 8 and Table 2, the effect of spinning distance on the size of gelatin nanofibers was not significant. However, lots of droplets on electrospun gelatin web were observed at farther distance (Fig. 9(b)).

To maintain a constant electric field (1.0 kV/cm), applied voltage should be controlled with a spinning distance together. In other words, higher electric field needs to be applied for farther distance. In this case, insufficiently charged droplets at the end of spinneret might be driven to collector. And it caused the existence of droplets on electrospun gelatin web. Therefore, proper conditions of electric field and spinning distance should be considered for the electrospinning. In case of gelatin nanofibers, these parameters may affect the size distribution and uniformity rather than mean size.

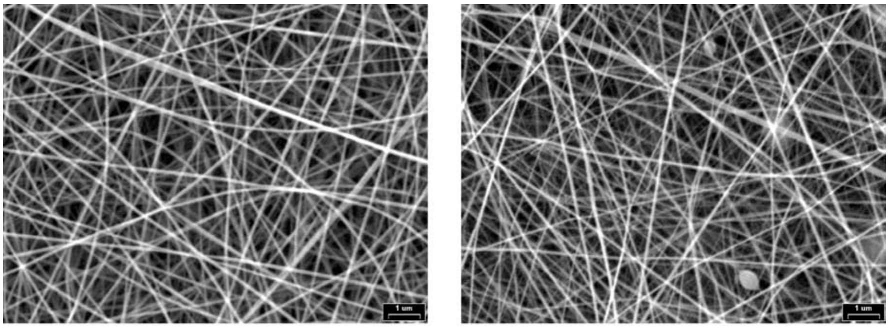
3.3. Characterization

3.3.1. FTIR spectroscopy

In order to investigate the possibility of structural change of gelatin molecules in formic acid, FTIR spectroscopy analysis was examined in the range of 1450–1750 cm^{-1}. Gelatin powder was used as a control but its spectrum was too complicated to be analyzed (Fig. 10(a)). And FTIR spectra of film cast from gelatin–formic acid solution and

Table 2
Electrospinnability and fiber diameter variation of gelatin on electric field and spinning distance

Electrospinnning conditions		Electrospinnability	Ave. diameter (nm)	Standard deviation (nm)
Electric field (kV/cm)	Distance (cm)			
0.75	10	Excellent	78	14
1.0	10	Excellent	76	14
2.0	10	Good (beads)	81	17
2.5	10	Poor (spray of droplets)	84	21
1.0	7.5	Excellent	79	14
1.0	10	Excellent	76	14
1.0	15	Good	95	22
1.0	20	Poor (beads and droplets)	81	16



(a) (b)

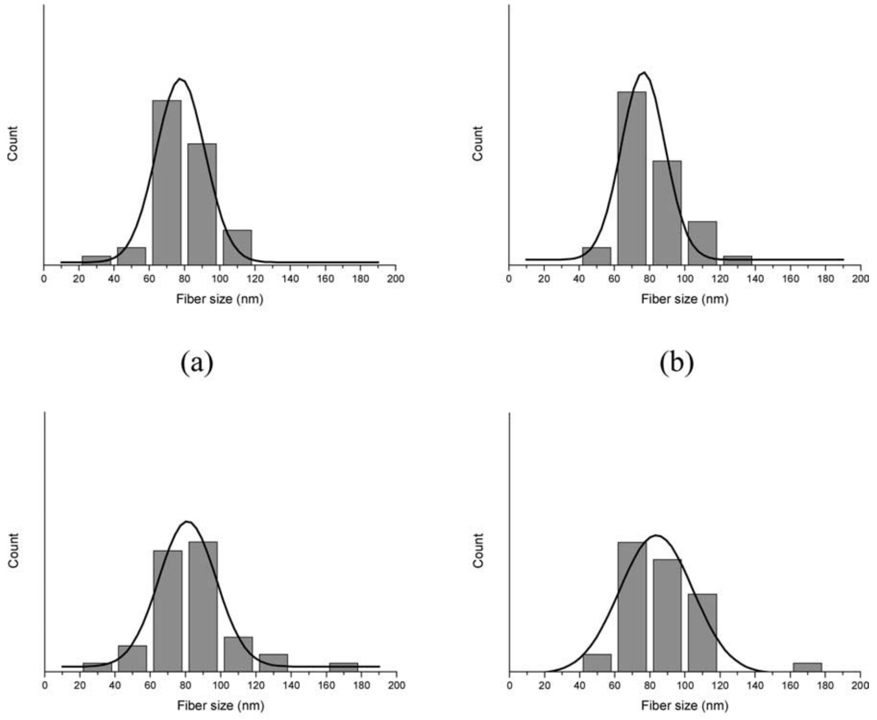
Fig. 6. Scanning electron microscope photographs of electrospun gelatin nanofibers varying electric fields (magnification $\times 10^4$); (a) 0.75 kV/cm, (b) 2.0 kV/cm.

electrospun gelatin web were compared with that of film cast from aqueous gelatin solution. As shown in Fig. 10, all samples illustrated common bands of protein appeared at approximately 1650 cm^{-1} (amide I) and 1540 cm^{-1} (amide II), corresponding stretching vibration of C=O bond, and coupling of bending of N–H bond and stretching of C–N bond, respectively [17,21]. The amide I band, at 1650 cm^{-1}, was attributable to both a random coil and α-helix conformation of gelatin reported by Prystupa et al. and Muyonga et al. [21,22].

There was little difference in FTIR spectra among the samples and it was hard to find out exact structural changes by FTIR analysis. Therefore, circular dichroism was performed for elucidating the conformational difference.

3.3.2. Circular dichroism

Fig. 11 shows CD spectra of electrospun nanofiber web and film prepared from gelatin–formic acid solution. These are also compared with the spectra of gelatin powder and film cast from aqueous gelatin solution. The spectra of all solutions exhibit two characteristic bands at the range of 200–210 and 220–225 nm. The negative band at the range



(a) (b) (c) (d)

Fig. 7. Size distribution of electrospun gelatin nanofibers under various electric fields at a fixed spinning distance of 10 cm; (a) 0.75 kV/cm, (b) 1.0 kV/cm, (c) 2.0 kV/cm and (d) 2.5 kV/cm.

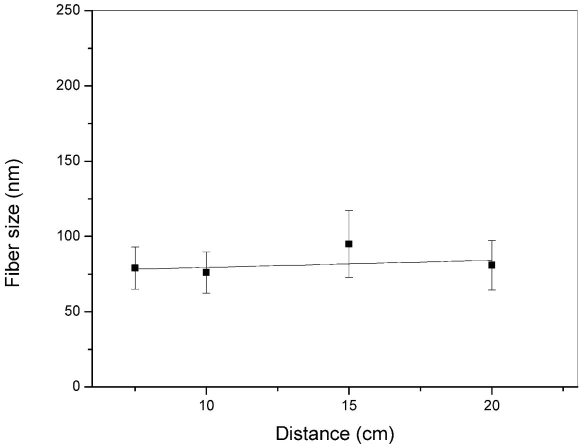


Fig. 8. Size of electrospun gelatin nanofibers at various spinning distances under electric field of 1.0 kV/cm.

of 200–210 nm and the slightly positive band at the range of 220–225 nm are known to attribute to a α-helix and triple-helix conformation, respectively. Even though these conformations are not clearly differentiated among the samples due to complicated nature in conformational structure of gelatin used in this study, the spectra of the gelatin powder and the film cast from aqueous gelatin solution (Fig. 11(a) and (b)) are different from those of electrospun web and film which are prepared using formic acid (Fig. 11(c) and (d)).

Based on the intensity of two characteristic bands of 200–210 and 220–225 nm, a helical conformation seems to be transformed to a random coil conformation when formic acid is used for the dissolution of gelatin. That is, formic acid may affect the conformational change of gelatin during the process. Particularly, the intensities of the spectra of the electrospun gelatin nanofiber were much smaller, indicating large contents of random coil structure contained in the electrospun specimen. It means the degradation occurred by formic acid have caused the structural transition of the gelatin molecules although the viscosity of the dope has been little changed during the electrospinning process.

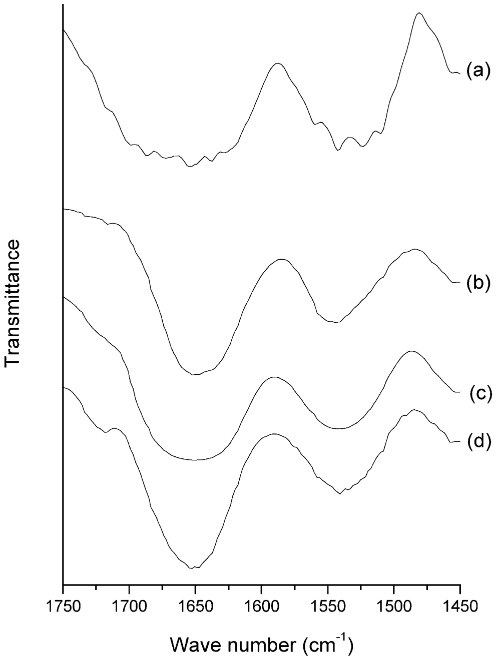
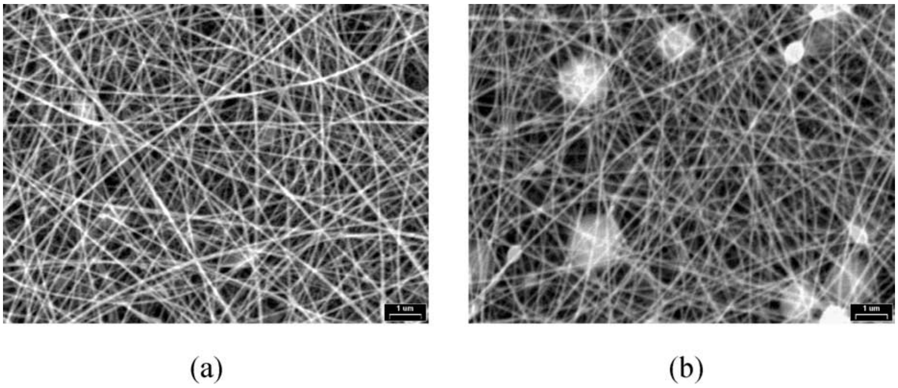


Fig. 10. FTIR spectra of (a) gelatin powder, (b) gelatin film cast from gelatin aqueous solution, (c) gelatin film cast from gelatin–formic acid solution and (c) electrospun gelatin nanofiber.

3.3.3. X-ray diffractometry

In order to examine the effect of formic acid on crystal structure and crystallinity of gelatin film and electrospun nanofiber, XRD patterns are investigated. As shown in Fig. 12(a), gelatin powder gives a typical XRD pattern of gelatin crystalline structure originated from α-helix and triple-helical structure. However, the film prepared from aqueous gelatin solution showed less developed crystal structure and lower crystallinity. Moreover, amorphous structures were observed for the film and electrospun nanofiber from gelatin–formic acid solution. The crystalline structure seems to be destroyed by regenerating the gelatin in formic acid. Even though α-helical structure was maintained in some extents (observed in CD data), very low crystallinity was observed from XRD pattern for the specimens prepared



(a) (b)

Fig. 9. Scanning electron microscope photographs of electrospun gelatin nanofibers at various spinning distances under electric field of 1.0 kV/cm (magnification $\times 10^4$); (a) 7.5 cm and (b) 20 cm.

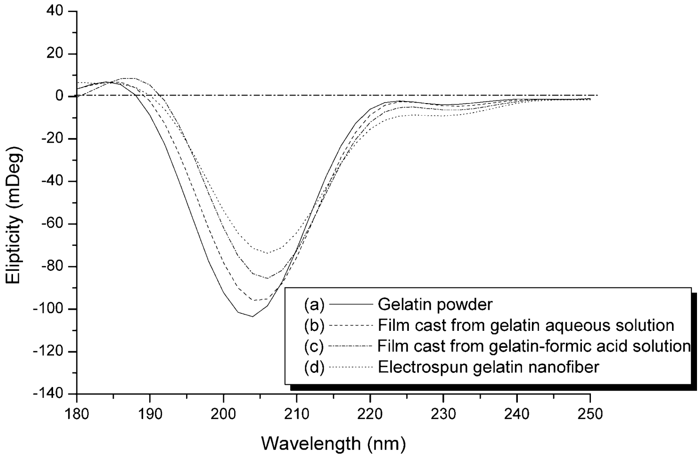


Fig. 11. Circular dichroism spectra of aqueous solutions of (a) gelatin powder, (b) gelatin film cast from gelatin aqueous solution, (c) gelatin film cast from gelatin–formic acid solution, and (d) electrospun gelatin nanofiber.

from gelatin–formic acid solution (Fig. 12(c) and (d)). The recrystallization of gelatin does not occur from formic acid solution (different from the case of fibroin [23]) and therefore, the gelatin nanofiber web becomes mostly amorphous with little crystallinity.

3.3.4. *Differential scanning calorimetry*

As shown in Fig. 13, the endothermic peak of raw gelatin powder appeared at 220 °C, which was known as a decomposition temperature of gelatin [18]. And the same endothermic peak was clearly observed for gelatin film cast from aqueous gelatin solution. However, in cases of film cast from gelatin–formic acid solution and electrospun gelatin nanofiber, there were no distinct thermal decomposition peaks. Only a broad trace of endotherm appeared at around 200 °C. It is attributed to the amorphous structure of random coil conformations and lower contents of helical conformations. This DSC results strongly support the XRD and CD data for these specimens.

4. Conclusion

Gelatin nanofibers were prepared by the electrospinning of gelatin–formic acid dope solution. Degradation of gelatin occurred in formic acid but this did not significantly affect the spinninability and morphology of electrospun gelatin nanofibers. Most of all, because formic acid has an advantage for dissolving the gelatin at room temperature and being very volatile, it can be used as a solvent for the electrospinning of gelatin. The electric field and spinning distance should be controlled properly for the preparation of gelatin nanofibers. Especially, the concentration was a major factor for the morphology by altering the size and

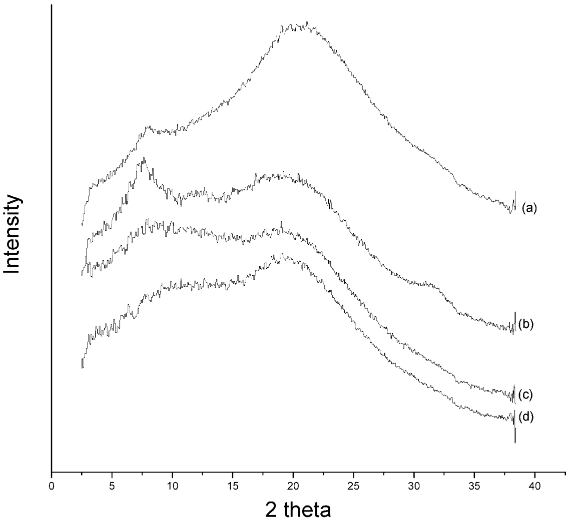


Fig. 12. X-ray diffractograms of (a) gelatin powder, (b) gelatin film cast from gelatin aqueous solution, (c) gelatin film cast from gelatin–formic acid solution, and (c) electrospun gelatin nanofiber.

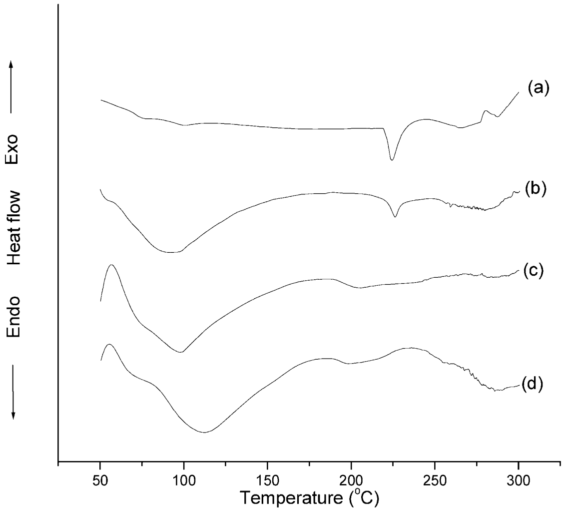


Fig. 13. DSC thermograms of (a) gelatin powder, (b) gelatin film cast from the gelatin aqueous solution, (c) gelatin film cast from gelatin–formic acid solution, and (c) electrospun gelatin nanofiber.

bead formation. Uniform and very fine gelatin nanofibers, varied from 70 to 170 nm, could be produced by controlling the dope concentration under an electric field of 1.0 kV/cm at the spinning distance of 10 cm.

As a result of structural analysis by FTIR spectroscopy and CD, the electrospun gelatin nanofiber showed a random coil and helical conformation. When formic acid was used for the dissolution of gelatin, structural transformation occurred from helical (α-helix and triple-helix) to random coil conformation in some extents. This related to amorphous nature of gelatin nanofibers prepared by the electrospinning of gelatin–formic acid solution. XRD and DSC analysis confirmed the amorphous structure and very low crystallinity, which is different from the specimen of original gelatin powder and gelatin film made from aqueous solution.

Acknowledgements

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