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Synthesis and characterization of nanofiber webs of chitosan/poly(vinyl alcohol) blends incorporated with silver nanoparticles

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Abstract Nanofiber webs of chitosan (CS)/poly(vinyl alcohol) (PVA) blends incorporated with silver nanoparticles (AgNs) were fabricated by two different methods: a refluxing method and an annealing method. We found that the characterization and antibacterial activity of AgNs depended on not only the fabrication methods but also the weight ratio of CS and PVA in the CS/PVA blend. The change in the size and number of AgNs due to the interaction between AgNs and CS, in turn, affected the antibacterial property of the non-woven webs. Non-woven webs of CS/PVA nanofibers containing AgNs that were fabricated by the refluxing method showed higher antibacterial ability against *Escherichia coli* than did the other types of non-woven webs. The morphology of the electrospun non-woven webs was observed by field emission scanning electron microscopy. The characterization of AgN formation on the surface of electrospun fibers was examined by transmission electron microscopy, attenuated total reflectance-Fourier transform infrared spectroscopy, and X-ray photoelectron spectroscopy.

Introduction

Electrospinning is a simple technique in which electrostatic forces are exploited to obtain nanofibers whose diameters vary from a few nanometers to several microns [1–3]. The electrospinning technique has attracted considerable attention, since it facilitates the synthesis of polymer electrospun (e-spun) fibers with unique properties, such as high porosity, small diameter, excellent pore interconnectivity, and high surface-to-volume ratio [4, 5]. As a result, this structure was found to improve certain characteristics of the materials. To functionalize the non-woven webs, small insoluble particles or soluble drugs can be added to the polymer solution and encapsulated in the nanofibers [2]. It has been reported that silver nanoparticles (AgNs) can be incorporated into e-spun fibers to obtain non-woven webs with high antibacterial activity [6–11].

AgNs are well known as effective antimicrobial and antifungal agents in comparison with other metals. The AgNs can attach to the cell membrane and penetrate the bacterial cell wall. Therein, the AgNs release Ag ions that can bind to tissue proteins and change the structure of the bacterial wall and nuclear membrane, leading to cell death [12–14]. The AgNs have an extremely large surface area, which provides increased contact with microorganisms, thereby enhancing their bactericidal activity. Owing to the unique characteristics of e-spun fibers, nanofiber webs incorporated with AgNs are ideal materials for use as antibacterial scaffolds. It was reported that the antimicrobial activity of nanofiber webs containing AgNs strongly depends on the size, shape, content, and distribution of AgNs in the e-spun fibers [15]. According to Son et al. [7], nanofiber webs of cellulose acetate containing AgNs with an average size of 21 nm successfully inhibited the growth of various types of bacteria. In their study, AgNs were produced by UV irradiation of cellulose acetate nanofibers, which were e-spun from a cellulose acetate solution of silver nitrate. Thus far, e-spun fibers containing AgNs have been prepared using many types of polymers. Poly(*N*-vinylpyrrolidone), poly(acrylonitrile), and polyurethane nanofibers containing AgNs have been fabricated for use in various applications, including antimicrobial filters [16, 17]. The

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fabrication was simplified using *N*,*N*-dimethylformamide as a solvent for these polymers and as reducing agent for Ag ions. Some natural biodegradable polymers have also been e-spun and incorporated with AgNs [9, 18]. Xu et al. [9] showed the antibacterial efficiency of the AgNs and the biodegradability of the poly(L-lactide) (PLA) composite nanofibers. They proposed that AgNs can be produced by electrospinning a PLA solution containing a small amount of silver nitrate and followed by hydrogen reduction. Although, currently, there is a limit to information concerning the effects of AgNs on general human health and the environment, the recent studies support the use of AgN-based materials in wound dressing, medical devices, tissue engineering scaffolds, textile fabrics, water treatment, etc. [5, 6]. However, some authors found that AgNs can cause impairment of mitochondrial function of cell [19, 20]. The potential cytotoxicity of AgNs may be strictly dependent on particle size, concentration, shape, the chemical and physical nature of the matrix, the site, and time of exposure [20, 21]. It was demonstrated that AgNs may induce cyto- and geno-toxic effects (cell death, DNA damage, and functional impairment) in human mesenchymal stem cells (hMSCs) at high exposure concentration, whereas antimicrobial effects of AgNs occur at much lower concentration [22]. Nevertheless, detailed studies of the hazardous effects of AgNs-based materials on the environment and human health need to be carried out.

Chitosan (CS), a (1-4)-linked 2-amino-2-deoxy-β-D-glucopyranose, is derived by the deacetylation of chitin [23, 24]. Among biopolymers, CS has been considered to be one of the most promising candidates for tissue-engineered scaffolds and wound dressing, owing to its excellent biological properties such as biodegradability, biocompatibility, antibacterial properties, and wound-healing activity [25, 26]. The antimicrobial activity of CS against yeast, fungi, and bacteria has been investigated in earlier studies [27, 28]. CS is known either to bind or modify minerals that are important for microbial growth or impair the membrane functions and inhibit bacterial replication by interacting with the cell membrane [29]. It was suggested that the potential antimicrobial activity of CS depends on many factors such as the deacetylation degree, molecular weight, and pH of the medium [30–32]. CSs markedly inhibited the growth of most bacteria tested, although their inhibitory effects differed according to molecular weight and the type of bacterium [32]. Previously, high antimicrobial activity of the CS–Ag^+ complex or the AgN-incorporated CS membrane has been reported by some authors [30, 33–35]. Velmurugan et al. [34] showed that the CS membrane is a good carrier for AgNs, and this AgN-incorporated membrane is highly active antifungal materials. Meanwhile, Chen et al. [30] demonstrated that the chelation of Ag ions significantly enhanced the antimicrobial activity of CS.

In this study, CS/poly(vinyl alcohol) (PVA) blend e-spun fibers containing AgNs were fabricated using two different methods: a refluxing method and an annealing method. In these non-woven webs, CS played a role not only as an antibacterial material but also as a carrier for AgNs because of its strong metal binding property. PVA is a non-toxic, biocompatible, and strong synthetic polymer, which is widely used in the biomedical field [23]. Moreover, PVA has good fiber-forming ability. Since it is not feasible to fabricate pure CS e-spun fibers, owing to its highly viscous solution and polycationic possession, electrospinning of the CS/PVA blend is an efficient way to enhance the electrospinnability of CS and improve the material properties such as high tensile strength. This study aims to investigate the effect of fabrication methods and the role of CS on the particularity and antibacterial property of nanofiber webs of CS/PVA blend incorporated with AgNs. The shape and size of AgNs and the interaction between the CS and AgNs on the surface of e-spun fibers were studied by transmission electron microscopy (TEM). The formation of AgNs and CS bonding were examined by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The morphology of e-spun blend fibers of the non-woven webs was investigated by field emission scanning electron microscopy (FE-SEM).

Materials and methods

Materials

PVA (88% hydrolyzed, $M_w = 88{,}000$) was purchased from Acros Organic Co. CS (deacetylation degree: 90%, $M_w = 690{,}000$) was supplied by Biomaterials Co (Korea). $AgNO_3$ (99.8%) and acetic acid (glacial 99.5%) were purchased from Samchun Co (Korea). Double-distilled water was used. All chemicals were used without further purification.

Fabrication of non-woven webs

In the refluxing method, 12 wt% aqueous PVA solution containing $AgNO_3$ was refluxed for 48 h. Then, the refluxed polymer solution was blended with 6 wt% CS solution (in 15 wt% acetic acid). The weight ratios of polymer solutions in the CS/PVA blend were 5.5/94.5 and 12.5/84.5. The amount of $AgNO_3$ added to the solutions was calculated from the percentage of the total weight of PVA and CS. These blended polymer solutions were then e-spun. In the annealing method, 12 wt% aqueous PVA solution containing $AgNO_3$ was blended with 6 wt% CS at the same ratio as that in the refluxing method. The e-spun

non-woven webs thus obtained were heat-annealed at 130 °C for 16 h to reduce the Ag ions in the e-spun fibers. Electrospinning was carried out using the blended polymer solutions. Each of the prepared solutions was poured into a standard 5-mL plastic syringe that was attached to a blunt 22-gauge stainless steel hypodermic needle. The solution flow rate was controlled using a syringe pump. A piece of aluminum sheet was wrapped around a rotating collector that was connected to the negative electrode. A high supply voltage (Chungpa EMI, Korea) was applied to the hypodermic needle as a positive electrode. The polymer solution was e-spun at a positive voltage of 16 kV, a needle tip-to-collector distance of 12 cm, and a solution flow rate of 5 μL/min.

Non-woven webs of PVA and CS/PVA nanofibers containing AgNs were cross linked using glutaraldehyde (GA) as a cross linker. The cross linking of the non-woven webs was carried out by immersing these non-woven webs in a solution of 1.25 wt% GA in acetone for 6 h at room temperature. A small amount of 96 wt% H_2SO_4 was added to the cross-linking solution as a catalyst. After immersion, the samples were thoroughly washed and rinsed with a copious amount of acetone to remove unreacted GA in the non-woven webs. The cross-linked non-woven webs were dried under vacuum.

Instrumentation

UV–Vis absorption spectra were obtained using a SpectraMax Plus384 (Molecular Devices, USA). TEM (TEM, TecnaiG^2, USA) was adopted to observe the AgNs on the surface of the e-spun fibers in the non-woven webs with carbon-coated copper grids. The morphology of the non-woven webs was determined by a FE-SEM (FE-SEM, HITACHI S-4700, Japan) using BAL-TEC MED020 system to place a conductive coating on the fibers before observing with a FE-SEM. From the FE-SEM and TEM photographs, the average diameter and diameter ranges of nanofibers and AgNs were measured using visualization software (TOMORO ScopeEye 3.6). The surface of the non-woven webs was examined using a Fourier transform infrared spectrometer (JASCO, ATR-FTIR 6100, Japan) and a MultiLab 2000 X- ray photoelectron spectrometer (XPS, Thermo VG Co, USA).

Antibacterial test

The antibacterial activity of the nanofiber webs was evaluated by testing them against a common bacterium, namely, *Escherichia coli* (Gram negative; ATCC 43895; *E. coli*) using the colony counting method. Small fragments of the nanofiber webs (approximately dimension of 6.0 × 3.0 cm and weight of 0.018 g) were introduced into Difco™ Nutrient Broth solutions with different bacterial concentrations. The mixtures were cultured at 37 °C in a shaking incubator for 15 h. Then, 100 μL of each of these solutions was seeded onto Sorbitol MacConkey agar by a surface spread plate technique. After incubation at 37 °C for 24 h, the number of bacterial colonies formed per milliliter (CFU/mL) was counted. This count was then used to calculate the surviving number of CFUs [36]. A pure PVA non-woven web was used in the test as a blank control. These agar plates were photographed for further evaluation.

Results and discussion

Fabrication of Ag nanoparticles by the refluxing method

In the refluxing method, AgNs were prepared by the reduction of silver nitrate in PVA solution. Then, the refluxed solution was blended with CS solution. The formation of AgNs in PVA solution before and after blending with CS solution at different refluxing time was monitored by UV–Vis spectroscopy (Fig. 1). It can be observed that all UV–Vis spectra display the peak at 430 nm corresponding to the surface plasmon resonance (SPR) absorption of AgNs, confirming the reduction of Ag ions and subsequent formation of AgNs in the PVA solution. When the refluxing time was increased, the intensities of SPR absorption of

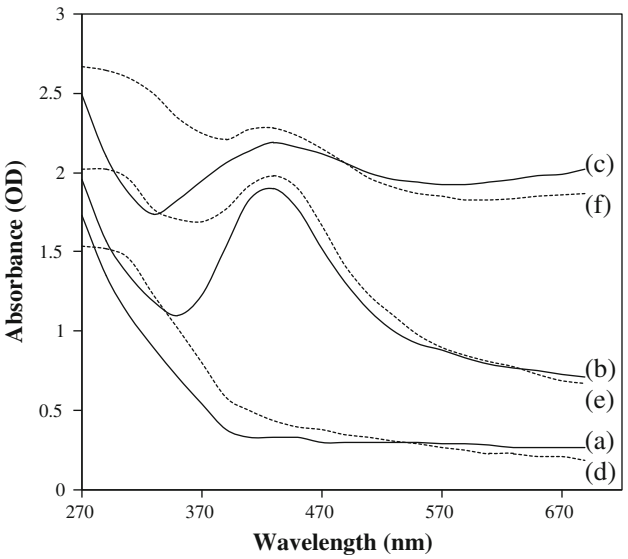

Fig. 1 UV–Vis absorption spectra of PVA aqueous solutions and the CS/PVA blend (12.5/87.5) solutions containing 2 wt% $AgNO_3$; each spectrum was obtained after the solution was refluxed for different lengths of time. The Ag/PVA solution was refluxed for **a** 0 h, **b** 12 h, and **c** 48 h, and the Ag/CS/PVA blend solution was refluxed for **d** 0 h, **e** 12 h, and **f** 48 h

AgNs increased, indicating that more AgNs were produced in the polymer solution. The positions of the peak maximum did not shift to a high wavelength with increasing refluxing time; this implies that the average sizes of the AgNs did not increase. Jin et al. [8] explained that PVA stabilized the AgNs through the coordination between the lone pair electrons on the hydroxyl oxygen of PVA with Ag ions and AgNs. Moreover, the PVA chains sterically inhibited particle nucleation and growth. The intensities of SPR absorption of AgNs in the CS/PVA blend solutions were slightly higher than those of AgNs in the PVA solution. However, the wavelength of AgN absorption did not change. This result indicates that CS played the role of both the stabilizing and reducing agents in the formation of AgNs, which is consistent with the result of Wei et al. [37] and Tran et al. [13].

Morphology of electrospun fibers

The morphology of the PVA and the CS/PVA blend e-spun fibers is observed in the FE-SEM images shown in Fig. 2. The average diameter of the e-spun PVA fibers was 714 nm, whereas that of e-spun CS/PVA blend fibers was considerably smaller. On increasing the CS content in the blend from 5.5/94.5 to 12.5/87.5, the average diameter decreased from 517 to 389 nm.

Figure 3 shows the effect of the presence of AgNs, Ag ions, and CS in the polymer solution on the morphology, average diameter, and diameter distribution of the nanofibers. The addition of AgNs and Ag ions was found to reduce the average diameters of nanofibers. Moreover, it was observed that the combination of CS and AgNs significantly decreased the diameter of e-spun fibers, leading to lower porosity of the non-woven webs, as shown in Fig. 3b, c, e, f. This behavior can be explained as follows. The stretching or drawing of the electrospinning jet is dependent on the ability of the solution to carry charges. Hence, the conductivity of the solution was found to change the morphology of the e-spun fibers significantly [23]. CS is a cationic polysaccharide with amino groups. Therefore, both AgNs and CS are ionic conductors in solution. The addition of AgNs, CS, or both increased the charge density in the ejected jets; thus, strong elongation forces were exerted on the jets under an electrical field, resulting in a small diameter [17]. There is no clear difference between the morphologies of the non-woven webs containing AgNs that were fabricated by the annealing method and the refluxing method.

Interaction between Ag nanoparticles and CS

Figure 4 shows the TEM images of e-spun fibers of PVA, the CS/PVA blend with a CS content of 5.5/94.5, and the

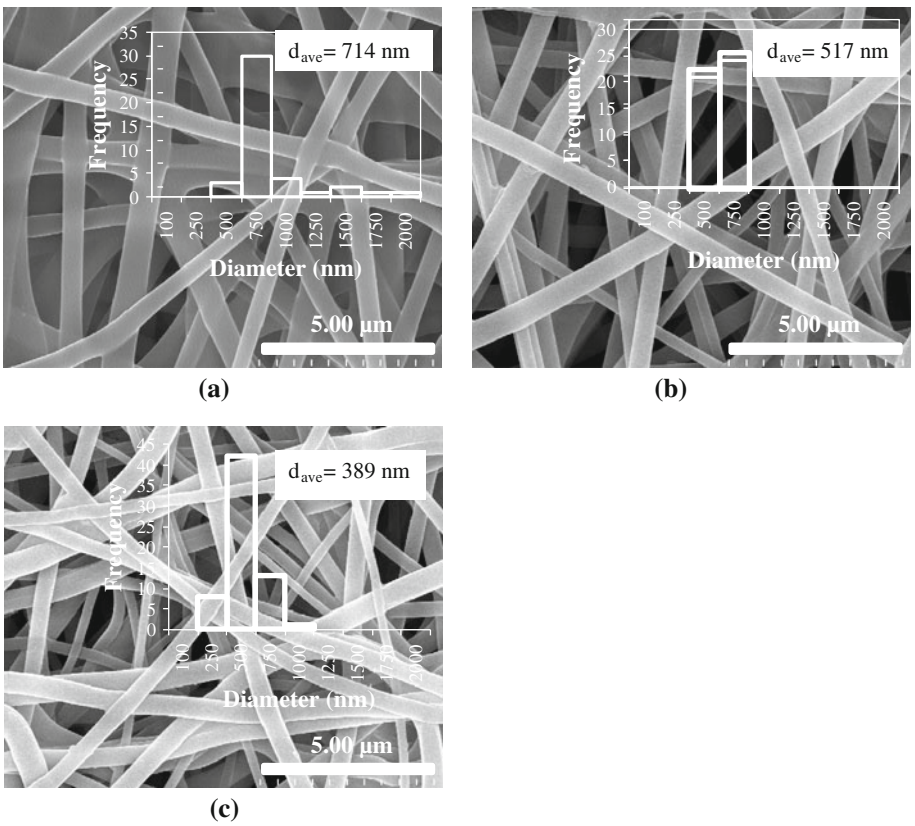

Fig. 2 FE-SEM images of non-woven webs of **a** PVA, **b** the CS/PVA blend with a CS content of 5.5/94.5, and **c** the CS/PVA blend with a CS content of 12.5/87.5 (the inserted graphs show the average diameter (d_{ave}) and diameter distribution of nanofibers)

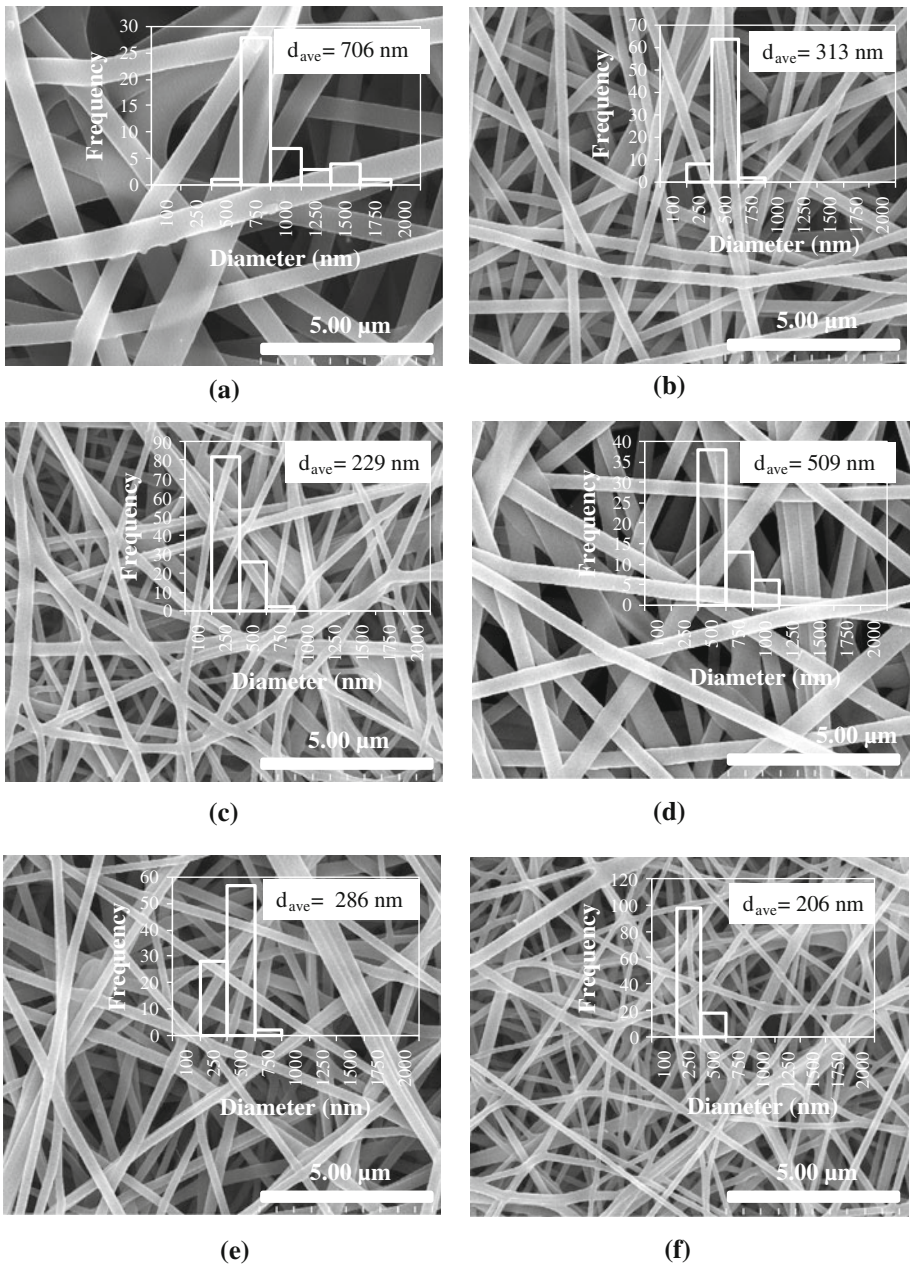

Fig. 3 FE-SEM images of non-woven webs containing AgNs fabricated by the annealing method: **a** Ag/PVA, **b** the Ag/CS/PVA blend with a CS content of 5.5/94.5, **c** the Ag/CS/PVA blend with a CS content of 12.5/87.5; images of non-woven webs containing AgNs fabricated by the refluxing method: **d** Ag/PVA, **e** the Ag/CS/PVA blend with a CS content of 5.5/94.5, **f** the Ag/CS/PVA blend with a CS content of 12.5/87.5 (the inserted graphs show the average diameter (d_{ave}) and diameter distribution of nanofibers)

CS/PVA blend with a CS content of 12.5/87.5 containing AgNs prepared by the annealing and refluxing methods. It was observed that the AgNs were all spherical and well distributed on the surface of the e-spun fibers. However, the number and size of AgNs varied according to the fabrication method and CS ratio in the blend. Using the refluxing method, a larger number of AgNs with smaller diameters could be formed on the surface of the e-spun fibers, compared to the case of the annealing method. This might be because of the difference in the state phase in which the AgNs were fabricated. In the refluxing method, the AgNs had relatively uniform and small size since they were formed in the solution by the reduction of Ag ions. On the other hand, in the annealing method, large AgNs were formed by the diffusion and agglomeration of the residual Ag ions and AgNs formed in the e-spun fibers (solid state) during heat treatment [8]. The presence of CS in the Ag/PVA solution significantly influenced not only the morphology of the non-woven webs but also the size and number of AgNs in the e-spun fibers. An increase in the CS content in the blends led to a reduction in the number of AgNs and an increase in their size. This phenomenon can be explained by the fact that AgNs were agglomerated into a cluster. According to the report of Tran et al. [13], the activation energy was found to be associated with the surface energy of the small Ag nuclei. The formation of Ag

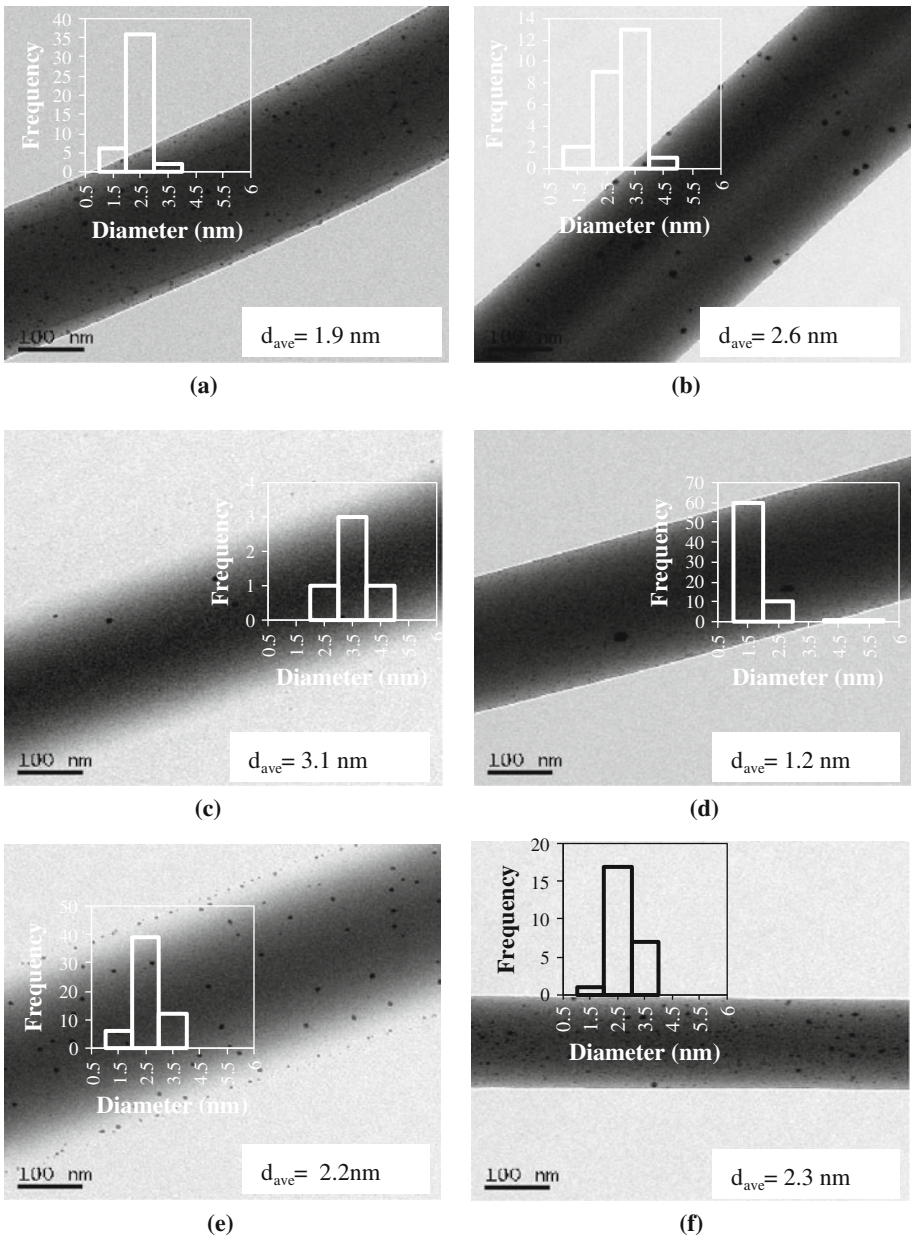

Fig. 4 TEM images of the e-spun fiber containing AgNs that was fabricated by the annealing method: **a** PVA, **b** the CS/PVA blend with a CS content of 5.5/94.5, and **c** the CS/PVA blend with a CS content of 12.5/87.5; TEM images of the e-spun fiber containing AgNs that was fabricated by the refluxing method: **d** PVA, **e** the CS/PVA blend with a CS content of 5.5/94.5, **f** the CS/PVA blend with a CS content of 12.5/87.5 (the inserted graphs show the average diameter (d_{ave}) and diameter distribution of AgNs)

atoms was stabilized by the CS polymer, and this was followed by agglomeration into clusters. Further, surface chelation of AgNs with CS molecules took place. The lone pair electron of the free amino group of CS molecules coordinated with AgNs. However, as inferred from the UV–Vis spectra, the peak maximum corresponding to AgNs in the blend solution did not shift significantly to longer wavelengths, indicating that this agglomeration did not occur during refluxing. On the basis of UV–Vis spectra and TEM results, it was assumed that the aggregation of AgNs occurred during electrospinning in the presence of CS. Under the electrical field, CS had higher potential of interaction with AgNs because of electrostatic attraction.

The formation of bonds between AgNs and CS is also demonstrated by ATR-FTIR spectra of non-woven webs of the CS/PVA blend and the Ag/CS/PVA blend nanofibers at the same ratio of CS/PVA of 12.5/87.5, prepared by the annealing and refluxing methods (Fig. 5). The ATR-FTIR spectra of the CS/PVA nanofiber webs show peak at 3300 cm^{-1}, which are characteristic of the combined peaks of the N–H and O–H group stretching vibration of CS. The presence of AgNs in the nanofiber webs not only decreased the transmittance but also shifted this band to higher wavenumbers, i.e., from 3300 to 3500 and 3450 cm^{-1} in the annealing and refluxing methods, respectively. This shows that binding of AgNs with the N of amine and O of

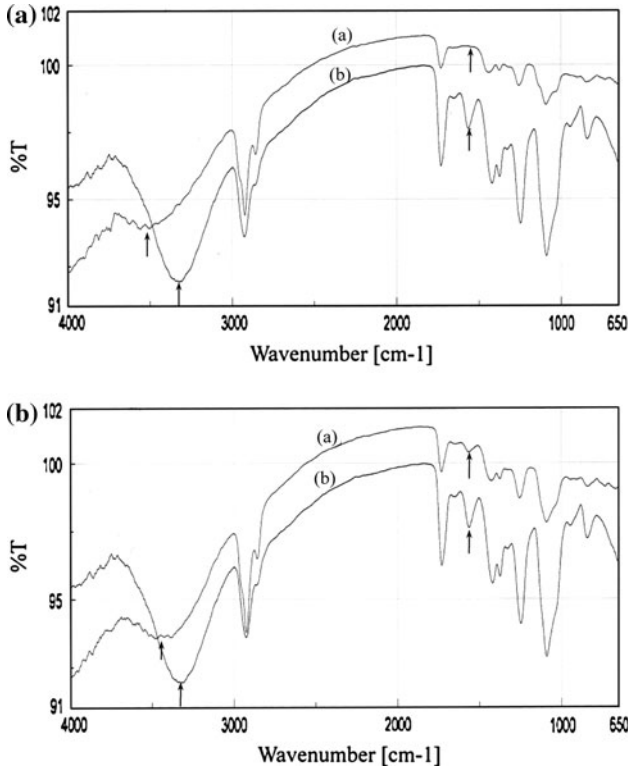

Fig. 5 ATR-FTIR spectra of non-woven webs of (*a*) the Ag/CS/PVA blend with a CS content of 12.5/87.5 and (*b*) the CS/PVA blend with a CS content of 12.5/87.5 fabricated by **a** the annealing method and **b** the refluxing method

hydroxyl group resulted in reduction amount of hydro bonding. In the case of the annealing method, the N–C=O group characterized by the vibration band at 1550 cm^{-1} disappeared, whereas in the refluxing method, this band significantly decreased the transmittance. This result can be explained by the attachment of AgNs to amino and amide groups. The existence of these bonds in the Ag/CS/PVA e-spun fibers resulted in strong linking between AgNs and CS molecules. This, in turn, led to the agglomeration of AgNs into a cluster that is shown in the TEM images in Fig. 4.

An XPS study was performed to characterize the Ag composition on the surface of non-woven webs. The Ag_{3d} scans of a non-woven web of Ag/PVA nanofibers and a non-woven web of Ag/CS/PVA blend nanofibers with CS content of 12.5/87.5 fabricated by the annealing and refluxing methods are shown in Fig. 6. The Ag_{3d} analysis confirmed the presence of AgNs and Ag ions on the surface of Ag/PVA non-woven webs fabricated by both methods (Fig. 6 A (a), B (a)). The Ag_{3d} peaks at 367.1 eV ($3d_{5/2}$) and 373.5 eV ($3d_{3/2}$) are characteristic of metallic Ag and residual Ag ions, respectively. However, these peaks are not observed in the Ag_{3d} photoemission spectra of non-woven webs of the Ag/CS/PVA blend nanofibers that were fabricated by both methods (Fig. 6 A (b), B (b)). It seems to be reasonable to state that the disappearance of the peaks that are characteristic of Ag and Ag ions was caused by the presence of CS. The bond formation between CS and AgN made a CS layer that covered around AgN. This conclusion is based on the principle of the XPS system. XPS spectra were obtained by irradiating a material with an X-ray beam while simultaneously measuring the kinetic energy and number of electrons that escape from the material surface. It was assumed that all the photo-emitted electrons from AgNs were either recaptured or trapped in various excited states within the material because of the CS sheath layer. Therefore, no electron emission from the surface of AgNs was detected by XPS, and the Ag_{3d} peak did not exist in this case. This core/sheath structure of AgNs and CS is further proof of interactions between them.

Antibacterial test

The antibacterial activities of the non-woven webs of CS/PVA and Ag/CS/PVA blend nanofibers against *E. coli* were studied using the viable cell counting method. Table 1

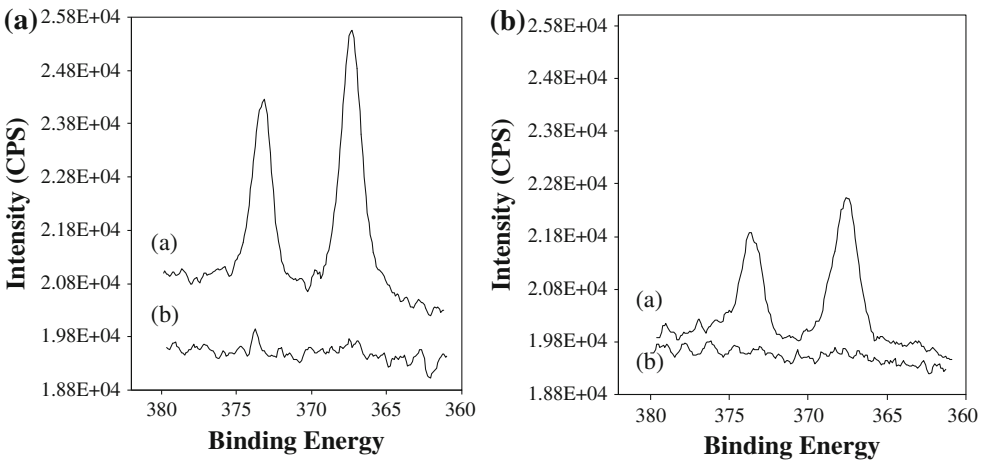

Fig. 6 XPS spectra (Ag_{3d} scan) of (*a*) a non-woven web of Ag/PVA and (*b*) non-woven webs of Ag/CS/PVA blend with a CS content of 12.5/87.5, fabricated by **a** the annealing method and **b** the refluxing method

Table 1 Results on the antibacterial activity of the non-woven webs against *E. coli*

Method	Bacterial concentration	CFU/mL after 15 h incubation				
		Ag/PVA	Ag/CS/PVA (5.5/94.5)	Ag/CS/PVA (12.5/87.5)	CS/PVA (5.5/94.5)	CS/PVA (12.5/87.5)
Blends	7×10^2	–	–	–	None	None
	7×10^5	–	–	–	250×10^1	None
Annealing	7×10^7	300	50	None	–	–
	7×10^8	271×10^3	201×10^2	118×10^2	–	–
Refluxing	7×10^7	None	None	None	–	–
	7×10^8	250×10^2	350	None	–	–

Non-woven webs of the CS/PVA blend nanofibers, Ag/PVA nanofibers, and the Ag/CS/PVA blend nanofibers were fabricated by the annealing and refluxing methods. The polymer solution contained 1 wt% $AgNO_3$, and the weight ratios of the CS/PVA blend were 5.5/94.5 and 12.5/87.5

presents the number of surviving *E. coli* cells on an agar plate after bacteria at different concentrations (7×10^7 and 7×10^8 CFU/mL) were incubated with a piece of the non-woven webs of Ag/CS/PVA nanofibers at 37 °C for 15 h. Figure 7 shows the corresponding photographs of surviving *E. coli* cells on an agar plate. The bactericidal effect of the non-woven webs was evaluated on the basis of the bacterial concentration at which all the bacteria were killed. It is worth emphasizing that the test samples showed an antibacterial effect that not only inhibited bacterial growth but also killed the bacteria. According to previous studies, CS itself shows antimicrobial activity. Our results showed that the bactericidal effect of the non-woven webs of the CS/PVA blend nanofibers increased with the CS content. The maximum concentration of bacteria that could be killed by the non-woven webs of the CS/PVA blend nanofibers at the ratio of 12.5/87.5 is 7×10^5 CFU/mL, while the non-woven webs of the CS/PVA blend nanofibers containing AgNs that were fabricated by the refluxing method showed higher antibacterial activity. This sample could kill all the bacteria at a maximum concentration of 7×10^8 CFU/mL. The difference between the antibacterial potentials of AgNs and CS could be caused by the direct interaction between these agents and the cell membranes of the bacteria. It is clear from Table 1 that in both the fabrication methods, the antibacterial activity of e-spun CS/PVA non-woven webs containing AgNs was higher than that of the e-spun non-woven webs without either CS or AgNs. These results also demonstrate that the antibacterial activity of the non-woven webs of Ag/CS/PVA nanofibers might involve the synergism of bactericidal effects of AgNs and CS.

Figure 7 shows that in comparison with the annealed samples, nanofibers webs prepared by the refluxing method could kill all the bacteria at a concentration of 7×10^7 (bacteria colonies were not observed). This indicates that the method used to reduce $AgNO_3$ to AgNs is effective. Ag/PVA nanofiber webs fabricated by the annealing method and refluxing method were separately added as antibacterial agents to a bacterial solution with a concentration of 7×10^7 CFU/mL that was incubated for 15 h; while 300 CFU/mL of *E. coli* remained in the former case, no bacterial colonies were found in the latter case. The same trend was also observed for the higher concentration of *E. coli* (7×10^8). Based on an antibacterial test and TEM results, it was suggested that the size of AgNs had some effects on the antibacterial activity; the smaller the AgNs, the stronger the antibacterial activity. The small size of the nanoparticles implies that they have a large surface that can contact with the bacterial cells, and hence, they will show a higher percentage of interactions than bigger particles [12]. This conclusion is consistent with those of some previous studies [15, 38].

Conclusions

Antimicrobial non-woven webs of the CS/PVA blend nanofibers containing AgNs were fabricated by electrospinning. AgNs were formed by the reduction of Ag ions in polymer solution before electrospinning by the refluxing method or by their reduction in e-spun fibers after electrospinning by the annealing method. The formation of AgNs in the polymer solution and the shape of AgNs on the surface of e-spun fibers were determined from UV–Vis spectra and TEM images, respectively. FE-SEM images showed the morphology of nanofiber webs, particularly, the changes in the diameter of the e-spun fibers in the non-woven webs, which depend on the CS/PVA ratio and whether AgNs are present. The interaction between the amino group of CS and AgNs in the e-spun fibers was confirmed by TEM, ATR-FTIR, and XPS analysis. This interaction was responsible for the formation of chelate, leading to the agglomeration of AgNs into a cluster covered by the CS layer. The e-spun fibers fabricated by the refluxing method contained larger number of AgNs with smaller size on their surface and showed higher antibacterial activity than those fabricated by the annealing method. Among the nanofiber webs of the CS/PVA blend,

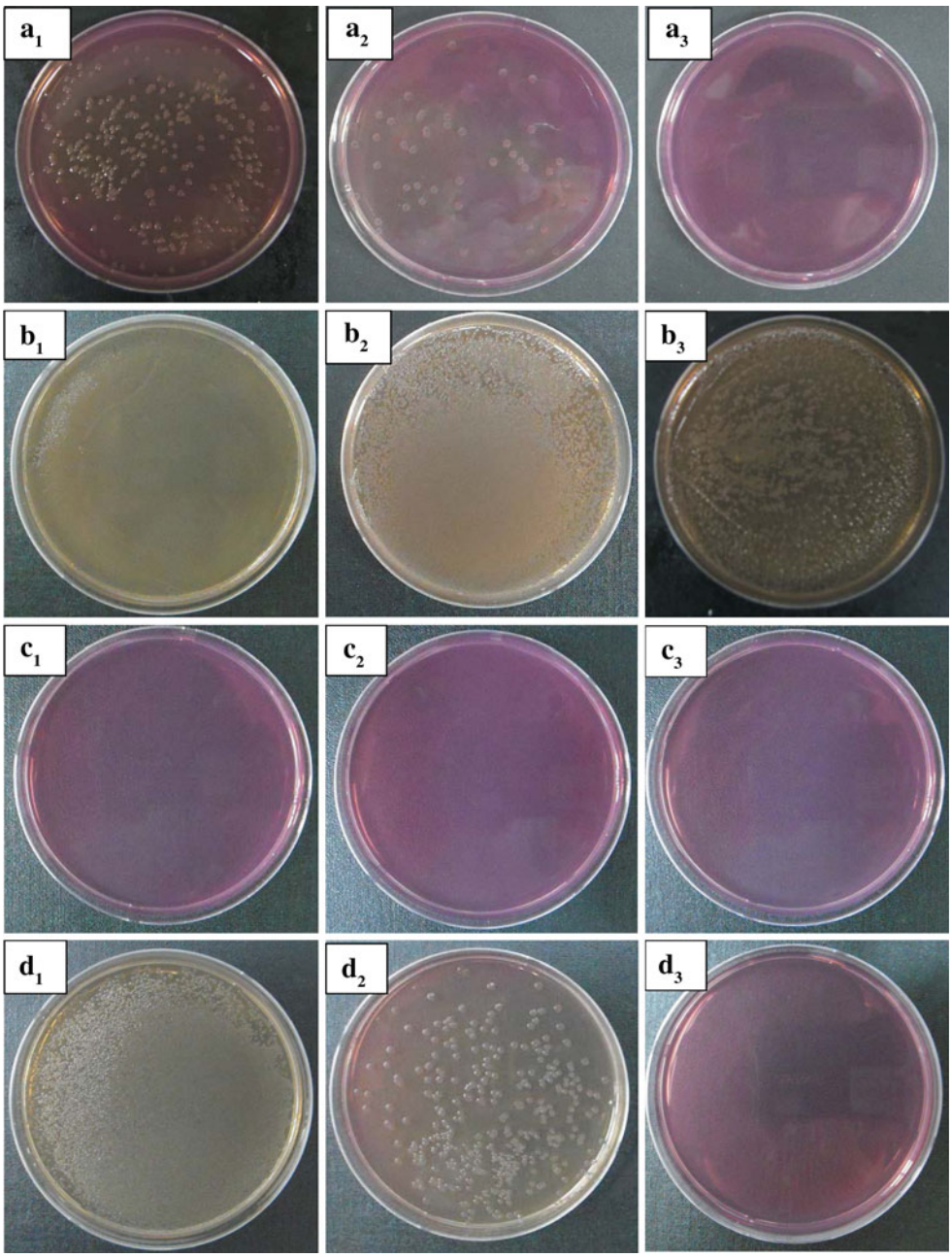

Fig. 7 Photographs show the surviving cells of *E. coli* on an agar plate after *E. coli* bacteria at different concentrations (7×10^7 and 7×10^8 CFU/mL) were incubated of bacterial with a piece of the non-woven webs of Ag/CS/PVA (approximately dimensions of 6.0×3.0 cm and weight of 0.018 g) at 37 °C for 15 h. Rows **a** and **b** show the antibacterial activity of the non-woven webs fabricated by the annealing method and refluxing method, respectively, when the concentration of *E. coli* was 7×10^7 CFU/mL. Rows **c** and **d** show the antibacterial activity of the non-woven webs fabricated by the annealing method and refluxing method, respectively, when the concentration of *E. coli* was 7×10^8 CFU/mL. The subscripts indicate the following: (*1*) non-woven webs of Ag/PVA nanofibers, (*2*) non-woven webs of Ag/CS/PVA blend nanofibers with a CS content of 5.5/94.5, and (*3*) non-woven webs of Ag/CS/PVA blend nanofibers with a CS content of 12.5/87.5

Ag/PVA, and CS/PVA blend containing AgNs, the third type of webs showed the highest antibacterial activity against *E. coli*. This is because of the synergism of antibacterial actions caused by the bactericidal effect of AgNs and that of CS. Owing to their high antimicrobial activity, these non-woven webs are promising candidates for use in medical sector. This application will require detailed studies of toxicity of the material.

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