

Preparation and Antibacterial Activity of Polyvinyl Alcohol/Regenerated Silk Fibroin Composite Fibers Containing Ag Nanoparticles

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ABSTRACT: Polyvinyl alcohol (PVA)/regenerated silk fibroin (SF)/AgNO₃ composite nanofibers were prepared by electrospinning. A large number of nanoparticles containing silver were generated *in situ* and well-dispersed nanoparticles were confirmed by transmission electron microscopy (TEM) intuitively. Ultraviolet (UV)-visible spectroscopy and X-ray diffraction (XRD) patterns indicated that nanoparticles containing Ag were present both in blend solution and in composite nanofibers after heat treatment and after subsequent UV irradiation. By annealing the nanofibers, Ag⁺ therein was reduced so as to produce nanoparticles containing silver. By combining heat treatment with UV irradiation, Ag⁺ was transformed

into Ag clusters and further oxidized into Ag₃O₄ and Ag₂O₂. Especially size of the nanoparticles increased with heat treatment and subsequent UV irradiation. This indicated that the nanoparticles containing silver could be regulated by heat treatment and UV irradiation. The antimicrobial activity of heat-treated composite nanofibers was evaluated by Halo test method and the resultant nanofibers showed very strong antimicrobial activity. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 123: 20–25, 2012

Key words: poly(vinyl alcohol); regenerated silk fibroin; electrospinning; Ag nanoparticles; nanofibers

INTRODUCTION

SF materials have been used to form a variety of biomaterials, such as gels,^{1,2} sponges,^{2,3} membranes,^{4,5} and films,² for medical applications. Recently electrospun SF fiber has been paid close attention to, because from a biological viewpoint, almost all of human tissues and organs are characterized by well organized hierarchical fibrous structures realigning in nanometer scale.^{5–7} However, the microbial contamination and poor mechanical properties of SF materials were two obstacles to their utilization.^{8,9}

The polymer blending technique can be considered to be a useful tool for the preparation of SF films or fibers with improved physical properties and performance. There were several articles on electrospinning of blends with SF.^{7,10–14} Regenerated silkworm

silk/polyethylene oxide blend nanofibers were prepared by electrospinning to study application of biomaterial matrices as scaffolds for tissue engineering.⁷ SF/chitosan electrospun nanofibers were prepared to investigate effect of chitosan on morphology and conformation of blend nanofibers.¹⁰ But until now, there have been no reports on the electrospinning of PVA/SF blends. PVA has good mechanical properties and potential biomedical applications,^{15,16} so the addition of PVA to SF could improve mechanical properties of SF and the SF added PVA should also have potential to be biomaterials.

Typical phase-separated structure was found in syndiotacticity-rich PVA and SF blend films.¹⁷ PVA and SF were incompatible after analyzing the structure and compatibility of PVA/SF films with deionized water as solvent.^{8,18} In our previous article,⁸ it was found that addition of glycerin could improve compatibility of SF and PVA with water as solvents, and mechanical properties of PVA/SF blend films increased after adding glycerin. The poor mechanical property of SF could also be overcome by improving processing techniques such as acidic spin dope systems,⁹ and ionic liquids had been used to successfully dissolve biological macromolecules.^{19–22} In this article, formic acid was applied as solvent of SF to improve compatibility of SF and PVA.

There is an increasing interest toward exploitation of silver nanoparticles technology in the

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development of nanobiomaterials, aiming at combining antibacterial property of silver with the peculiar performance of the biomaterials.^{23–25} The hydroxyl groups deployed on PVA chains could generate hydrogen bonds with many anions, which could enhance the solubility of silver salt in PVA solution.²⁶ By changing Ag^+ into metallic silver or its oxide in electrospun polymer fibers, final polymer nanofibers could exhibit distinctive properties of silver nanoparticles. In this article, formic acid was used to dissolve SF and improve compatibility of SF and PVA. PVA/SF/AgNO₃ composite nanofibers were prepared by electrospinning technology. Two posttreatment methods were adopted to regulate the size of nanoparticles containing silver in electrospun composite fibers. The antibacterial activity of final composite nanofibers was investigated.

EXPERIMENTAL

Preparation of electrospinning solution

Bombyx mori silk was degummed with 0.5 wt% Na₂CO₃ for 1 h at 100°C, and then rinsed thoroughly with deionized water. The degummed silk was dissolved in CaCl₂/H₂O/C₂H₅OH (1 : 8 : 2 in molar ratio) ternary solvent for 2 to 3 days, stirred the mixed solution was stirred at 60°C for 4 h to be dissolved completely. The obtained solution was dialyzed in deionized water for 3 to 4 days and centrifuged. The SF solid obtained after drying was dissolved in formic acid (>90%, Shanghai Chemical Reagent Factory, China) at 60°C for 4 h. Weight fraction of SF in SF/formic acid solution was 13 wt%. PVA (Sino-pharm Chemical Reagent Co., China) was dissolved in deionized water at 80°C for 4 h. Concentration of PVA aqueous solution was 7 wt%. The PVA solution and the SF/formic acid solution were mixed on the basis of mass ratios of PVA to SF which were varied from 90/10 to 70/30, after the mixture was stirred at 60°C for 4 h, silver nitrate (Shanghai Institute of Fine Chemicals, China) was added into it and then the mixture was stirred at 10°C for 20 h. The resulted mass ratio of silver in the silver nitrate to the total solid content in the resultant solution was 1–2 wt%.

Preparation of composite nanofibers

PVA/SF/AgNO₃ composite nanofibers were prepared by electrospinning the solutions stated above. The electrospinning setup used in this study consisted of a syringe, a plastic tip, a grounded collecting plate, and a high voltage supply.²⁷ The spinning solution was electrospun at 20 KV, with tip-to-collector distance 18 cm. All electrospinning procedures were carried out at room temperature.

Posttreatment of composite nanofibers

To stabilize PVA/SF/AgNO₃ composite nanofibers²⁸ and regulate size of nanoparticles, composite nanofibers were placed in thermostat drying oven at 155°C for 5 min. On the other hand, UV irradiation ($\lambda = 365 \text{ nm}$, $P = 10 \text{ W}$) was used 3h for photoreduction treatment.

Measurements and characterization

Morphology of composite nanofibers was observed by using SEM (Hitachi S-570, Japan) after applying a gold coating. Average diameter of nanofibers was determined by analyzing SEM images using Photoshop analysis program. TEM images were obtained with a FEI TecnaiG 200 TEM using the samples deposited on carbon coated copper grids. Average size of nanoparticles was determined by analyzing TEM images using Photoshop analysis program. XRD of composite nanofibers after heat treatment and UV irradiation was measured with an X'Pert-Pro MPD diffractometer (PANalytical, Holland). UV-visible absorption spectra of blend solution were obtained with a U-3010 spectrometer (Hitachi, Japan) and in the wavelength region from 350 to 600 nm. Antimicrobial activities of composite nanofibers after heat treatment were tested against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8099). Agar plates containing test samples and control (blank) were incubated at certain temperature (*S. aureus*: 37°C; *E. coli*: 30°C) for 16 to 18 h.

RESULTS AND DISCUSSION

Structural characterization

The incorporation of metal nanoparticles into polymer nanofibers can be achieved using either electrospinning polymer solution containing metal nanoparticles or reducing metal salts or complexes in electrospun polymer nanofibers.^{29–32} In this study, PVA/SF composite fibers containing nanoparticles were prepared by latter method. Ionization free energy of Ag and the redox potential of Ag^+/Ag couple could be given by three methods: thermodynamic perturbation, overlapping distribution, and self-consistent histograms.³³ And a large number of reports were available on synthesis of nanoparticles containing Ag by different methods such as chemicals,^{32,34–36} UV irradiation,^{28,35,37} ultrasound,^{38,39} prolonged reflux,⁴⁰ γ -ray irradiation,⁴¹ and a simple heat treatment.²⁸ The common oven heat treatment and UV irradiation treatment were safe, convenient and effectual. So these two treatments were adopted to generate nanoparticles containing silver in this work. The functional Ag porous films were obtained by heat-treating AgNO₃-doped PVA electrospun

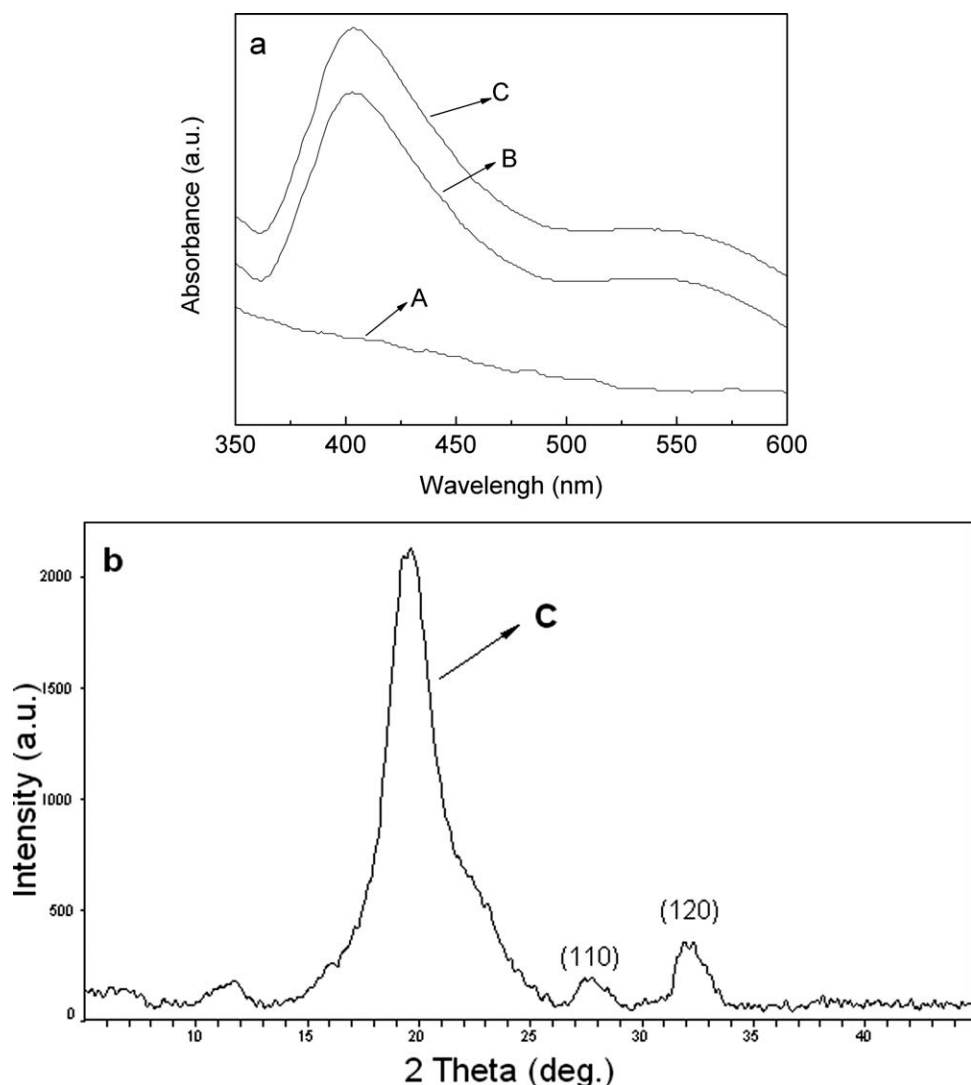


Figure 1 UV-visible absorption spectra of PVA/SF (80/20) composite solution (a) and X-ray diffraction patterns of PVA/SF (80/20) composite nanofibers (b) containing 2 wt% silver: (A) in the absence of silver, (B) after heat treatment, and (C) after subsequent UV irradiation.

nanofibers. But after heat treatment at 600°C for 2 h in air, PVA in nanofibers had degraded completely.⁴² The antimicrobial PVA nanofibers containing silver nanoparticles were prepared by electrospinning PVA/AgNO₃ aqueous solutions, followed by the heat treatment at 155°C for 3 min. Nanoparticles formed by heat treatment were homogeneously dispersed in PVA matrix and heat-treated nanofibers could preserve the web structure in water,²⁸ which is one of the significant conditions to wound-dressing biomaterials. In this study, composite nanofibers were heat-treated at 155°C for 5 min and subsequent UV irradiated at 365 nm for 3 h to form spherical and dispersed nanoparticles containing silver.

UV-visible absorption spectra of PVA/SF/AgNO₃ blend solution without silver and with 2 wt% silver after heat treatment and after subsequent UV irradiation are shown in Figure 1(a). A typical surface plasmon resonance band of silver at 403 nm is

observed for nanoparticles containing Ag in the mixture after heat treatment and after subsequent UV irradiation²³ [Fig. 1(a(B,C))]. These results confirm that Ag⁺ could be reduced by heat-treating and UV-irradiating. To analyze the composition of nanoparticles further, XRD pattern of the composite nanofibers after subsequent UV irradiation is measured [Fig. 1(b)]. The peak at $2\theta = 27.7^\circ$ is the peak corresponding to the indices (110) for Ag₃O₄. The peak at $2\theta = 32.5^\circ$ is the peak corresponding to the indices (120) for Ag₂O₂. These results show that during heat treatment and UV irradiation, Ag⁺ could be reduced into Ag clusters and further oxidized into Ag₃O₄ and Ag₂O₂.

Morphological characterization

Generation of nanoparticles containing Ag could be identified from the color change of the as-prepared

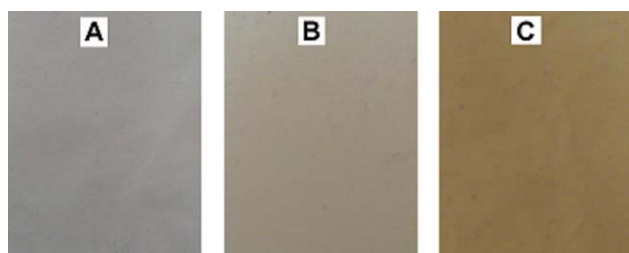


Figure 2 Images of PVA/SF (80/20) composite nanofiber webs containing 2 wt% silver: (A) without posttreatment, (B) after heat treatment, and (C) after subsequent UV irradiation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

products.⁴³ Figure 2 are images of PVA/SF/AgNO₃ composite nanofiber webs containing 2 wt% silver without post-treatment, with heat treatment and with subsequent UV irradiation. The color of webs changes from white to light yellow after heat treatment [Fig. 2(A,B)]. After subsequent UV irradiation, the color becomes tawny [Fig. 2(C)]. This indicates that Ag⁺ in the nanofibers is reduced and aggregated into nanoparticles containing silver during heat treatment and subsequent UV irradiation. Ag⁺ could not be reduced completely and still exists in composite nanofibers.

Heat-treated temperature (155°C) is higher than the boiling point of formic acid (100.8°C) and deion-

ized water (100°C). So the residual solvents should be volatilized during heat treatment. Figure 3 shows the typical SEM images and diameter distributions of composite nanofibers before and after heat treatment and after subsequent UV irradiation. The compatibility of PVA and SF improves with formic acid as solvent of SF. The average diameter of composite nanofibers is 266 nm without any posttreatment, this value decreases into 194 nm after heat treatment and decreases further into 152 nm after UV irradiation [Fig. 3(A–C)]. This demonstrates that the residual solvent which had not been volatilized during electrospinning process is volatilized during heat treatment and UV irradiation.

Figure 4 shows the typical TEM images of PVA/SF composite nanofibers and size distribution of nanoparticles before and after heat treatment and after subsequent UV irradiation. Nanoparticles exist in fiber without posttreatment, as shown in Figure 4(A). However, a number of nanoparticles which formed during electrospinning process and without posttreatment could not be observed on electrospun fibers of polyvinylpyrrolidone³⁵ and polyacrylonitrile,³⁶ After AgNO₃ solution without polymer was UV-irradiated for 36 h, there were no silver absorption band characteristic of surface plasmon resonance.⁴³ It might be due to the coordination between PVA and Ag⁺, which could decrease the potential of

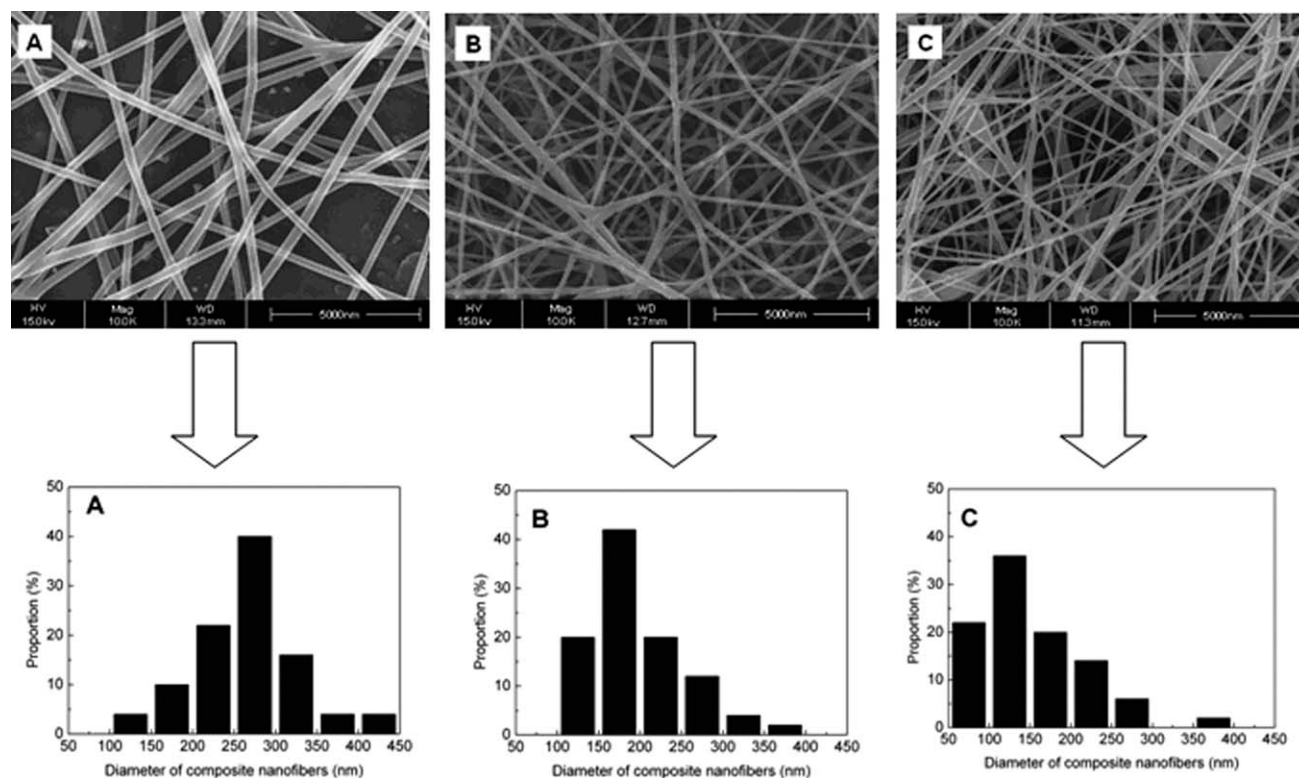


Figure 3 Typical SEM images and diameter distribution of PVA/SF (80/20) composite nanofibers containing 2 wt% silver: (A) without posttreatment, (B) after heat treatment, and (C) after subsequent UV irradiation.

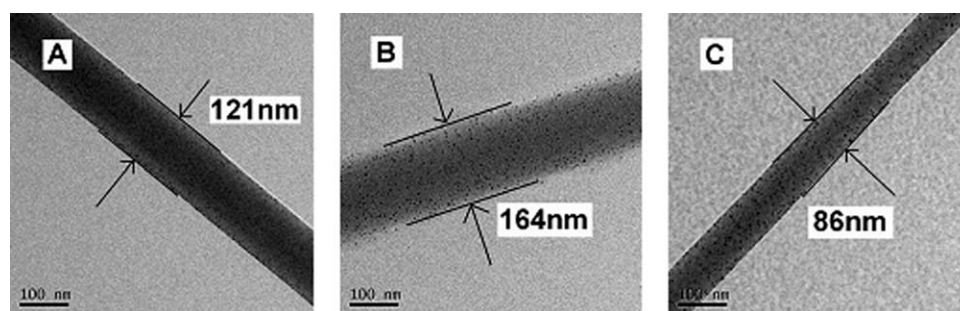


Figure 4 Typical TEM images of PVA/SF (80/20) composite nanofibers containing 1 wt% silver and the size distribution of nanoparticles: (A) without posttreatment, (B) after heat treatment, and (C) after subsequent UV irradiation.

Ag^+/Ag ($E_{\text{Ag}^+/\text{Ag}}$) and promote the reduction of Ag^+ . Therefore, PVA could be used as reducing agent of nanoparticles containing silver.

The shape of the nanoparticles was sphere and the size distributions of the nanoparticles were narrow. Average size of nanoparticles without posttreatment was 2.1 nm [Fig. 4(A)]. After heat treatment, average size increased to 2.4 nm [Fig. 4(B)] and this value increased to 3.5 nm after subsequent UV irradiation [Fig. 4(C)]. This indicates that after heat treatment and after subsequent UV irradiation, more Ag^+ is reduced into nanoparticles and then occur on fibers. Nanoparticles could aggregate together to generate larger particles.

Antimicrobial activity

The antimicrobial activity of silver enhanced with increasing silver chemical valence.⁴⁴ Ag_3O_4 and Ag_2O_2 were generated in fibers after heat treatment and subsequent UV irradiation in this article. So the composite nanofiber webs should exhibit strong antimicrobial activity. The heat-treated composite nanofiber webs were placed in bacteria-inoculated agar plates and visualized for antibacterial activity after being incubated for 16 to 18 h. The bacteria spread on agar plates resembled real world situations where the pathogenic bacteria are often present on receptive nutrient surfaces in biomedical implants, medical devices, or food packaging surfaces. The samples

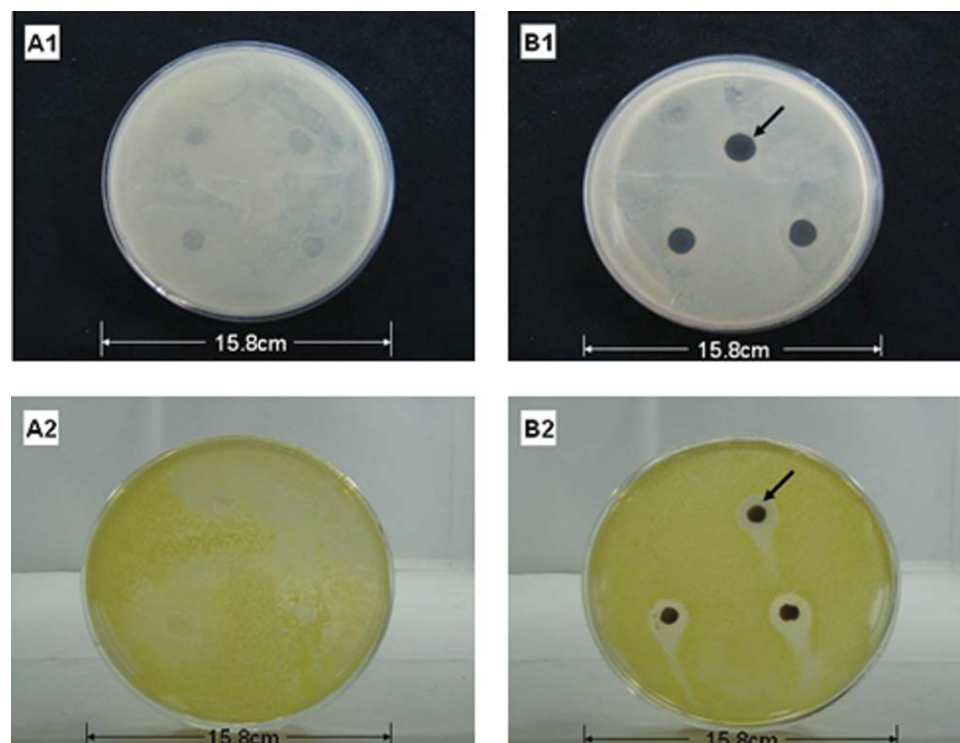


Figure 5 Test results of antimicrobial activities of the heat-treated PVA/SF (90/10) composite nanofibers. Zone of inhibition is indicated by arrows. (A) Without silver, and (B) with 2 wt% silver (A1, B1: *E. coli*. A2, B2: *S. aureus*). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://www.interscience.wiley.com).]

placed in the bacteria-inoculated agar plates killed all the bacteria over and around them. We observe that there is no zones of inhibition around the composite nanofiber webs without silver for both *E. coli* [Fig. 5(A1)] and *S. aureus* [Fig. 5(A2)], but there is evident zones of inhibition around the composite nanofiber webs with 2 wt% silver for both *E. coli* [Fig. 5(B1)] and *S. aureus* [Fig. 5(B2)]. The heat-treated composite nanofibers successfully inhibit the growth of the bacteria and had strong antimicrobial activity on *E. coli* and *S. aureus*.

CONCLUSIONS

The compatibility of PVA and SF improved distinctly with formic acid as solvent of SF. PVA/SF/AgNO₃ composite nanofibers were successfully prepared by electrospinning process. After an oven heat treatment at 155°C for 5 min, a large number of nanoparticles containing silver were generated throughout the nanofibers. Average diameter of composite nanofibers decreased with heat treatment and subsequent UV irradiation for 3 h, but size of nanoparticles in composite nanofibers increased after heat treatment and increased further after subsequent UV irradiation. Size of nanoparticles containing silver could be modulated by heat treatment and UV irradiation. The nanoparticles in the fibers after treatments were considered to consist of Ag₃O₄ and Ag₂O₂. Heat-treated composite fibers showed strong antimicrobial activity.

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