Antibacterial Electrospun Chitosan/Poly(vinyl alcohol) Nanofibers Containing Silver Nitrate and Titanium Dioxide

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ABSTRACT: Chitosan/poly(vinyl alcohol) (PVA) nanofibers with antibacterial activity were prepared by the electrospinning of a chitosan/PVA solution with a small amount of silver nitrate ($AgNO_3$) and titanium dioxide (TiO_2). Nanofibers with diameters of 270–360 nm were obtained. The yield of low-viscosity chitosan (LCS)/PVA nanofibers was higher than that of high-viscosity chitosan (HCS)/PVA, and the water content of the HCS/PVA nanofibers and the LCS/PVA nanofibers were 430 and 390%, respectively. The nanofibers developed in this study exhibited antibacterial activities of 99 and 98% against *Staphylococcus aureus* and *Escherichia coli*, respectively. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2892–2899, 2009

Key words: biodegradable; fibers; nanotechnology

INTRODUCTION

The antibacterial treatment of fibers started with the antibacterial treatment of fabric for protecting mummies in Egypt 4000 years ago. Recently, new antibacterial reagents and a mechanism for antibacterial activity on bacteria have been reported.[1–4]

Typical antibacterial treatment on a fiber uses natural materials having antibacterial properties and chemicals such as tertiary ammonium. The former is primarily for fibers mixed with chitosan.[5] Chitosan, however, has problems with durability and performance. Therefore, to improve the durability of the chitosan, chitosan–cellulose fibers have been developed by a melt-spinning method. Although the melt-spun fibers have a good durability, they give rise to lower performance than chitosan-coated fibers. Thus, a recent approach for the production of electrospun chitosan nanofibers was presented.[6(a)]

There have been few studies on nanofibers with a viewpoint of antibacterial application because of the limitation of its mass production, although Formhals submitted a patent for the manufacture of the nanofibers by electrospinning in 1934.[6(b)] The study of nanofibers, however, has rapidly increased with the development of nanotechnology starting at the end of 1990s. Although the melt-spinning method only makes fibers with diameters of around a few micrometers, nanofibers with a nanosized diameters have been developed to have very high specific surface areas.[7–16]

The electrospinning method is convenient to make nanofibers that have fiber diameters in the range of 500 nm or a few micrometers. Nanofibers have been studied because of their very small pore size, large porosity, and high specific surface area.[17] Electrospun nanofibers have recently been investigated for biomedical applications such as drug delivery and dressing materials for curing injury. In particular, blended polymers have come to occupy an important position in the development of functional nanofibers. Many researchers have focused on the electrospinning of blended polymer solutions of chitosan and poly(vinyl alcohol) (PVA) to make nanofibers as biomedical materials.[17]

In this article, we present the preparation of chitosan/PVA nanofibers having antibacterial activity with the addition of the antibacterial reagents silver nitrate ($AgNO_3$) and titanium dioxide (TiO_2). Several experimental factors for the efficient fabrication of nanofibers and their inherent antibacterial activities were also investigated.

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EXPERIMENTAL

Materials

Chitosan with two viscosities [low-viscosity chitosan (LCS) and high-viscosity chitosan (HCS)] was supplied by Samsung Chitopia Co., Ltd. (Siheung, Korea) and PVA (weight-average molecular weight = 44,000) was purchased from Hanawa Chemical Pure Co. (Osaka, Japan). Purified TiO_2 100 nm in size and $AgNO_3$ (assay: 99.8%) were purchased from Sigma Aldrich Chemical Co. Formic acid (Samchun Pure Chemical Co., Pyongtaek, Korea) was used for the electrospinning solvent, and all other reagents were used without any purification.

Electrospinning

The viscosity of the chitosan was measured with a conical rotational viscometer (Visconic EHD, Tokimec, Inc., Tokyo, Japan), which was used to determine the conditions of the electrospinning with two viscosities of the chitosan (LCS = 50 cPs, HCS = 1300 cPs). The chitosan was dissolved in formic acid (5 wt %) and stirred for 24 h at room temperature (25°C), and then, we measured its viscosity. To prepare the electrospinning solution, $AgNO_3$, TiO_2, and PVA were added to the chitosan solution at different ratios, as shown in Table I.

Figure 1 shows the electrospinning apparatus used to make the nanofibers, which consisted of a syringe pump, a high-voltage power supplier, and a collector. A high positive voltage was applied to the syringe, and the collector was grounded. The chitosan solution was instantly charged and electrospun to the collector surface with a voltage between 9 and 18 kV and a tip-to-collector distance (TCD) in the range 7.5–15.0 cm. The electrospun web was dried at 110°C for 12 h.

The production yield of the chitosan nanofibers was calculated by the following equation:

$$\text{Yield} = \frac{W_c}{W_f} \times 100 \quad (1)$$

where W_c is the weight of the chitosan and W_f is the weight of the nanofibers produced in the electrospinning process.

TABLE I
Electrospinning Conditions of Chitosan/PVA

Chitosan/PVA ratio (wt % : wt %)	Voltage (kV)	TCD (cm)	Flow rate (mL/h)
3 : 97	15	10	1.0
6 : 94	15	10	1.0
9 : 91	9–18	7.5–15	0.5–2.0
12 : 88	15	10	1.0
15 : 85	15	10	1.0

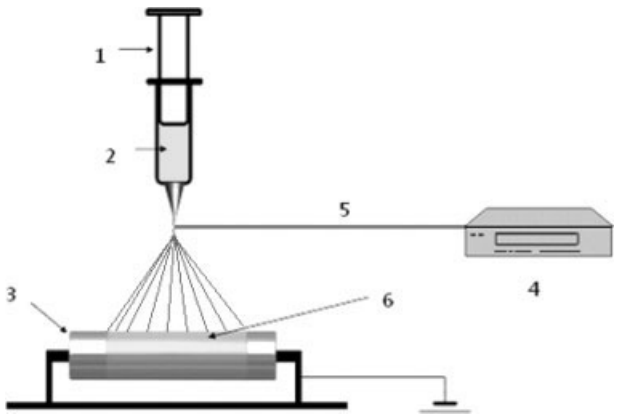

Figure 1 Schematic diagram of the electrospinning apparatus: (1) syringe pump, (2) electrospinning solution, (3) collector, (4) power supply, (5) electrical wire, and (6) nanofiber.

Water content

The dried electrospun web was weighed and immersed in distilled water for 24 h. Then, the excess water on the sample surface was quickly removed by blotting paper. The water content of the electrospun chitosan nanofibers with inorganic particles was calculated as follows:

$$\text{Water content } (\%) = 100 \times \left(\frac{w_{\text{wet}} - w_{\text{dry}}}{w_{\text{dry}}}\right) \quad (2)$$

where w_{wet} is the wet weight of the electrospun fiber and w_{dry} is its dry weight.

Analysis

The chemical structure of the chitosan nanofibers was analyzed by Fourier transform infrared (FTIR) spectroscopy (Shimadzu, IR Prestige 21, Kyoto, Japan) with a frequency in the range 4000–600 cm^{-1}, a scan number of 20 times, and a resolution of 4 cm^{-1}. The morphology of the chitosan nanofibers was observed by scanning electron microscopy (SEM; Simens, LEO 1455VP, Nuremberg, Germany) after gold coating.

Antibacterial test

The shake flask method was used to examine the antibacterial activity of the electrospun chitosan/PVA nanofibers. Gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* were selected as representative microorganisms, and nutrient agar supplied by Difco Co. (Difco Laboratory, MD) was used as a culture medium. To investigate the antibacterial activity, the nanofibers were loaded in a bacterial culture medium and were stirred for a specific time. Then, the number of bacteria was indirectly measured by optical density in an

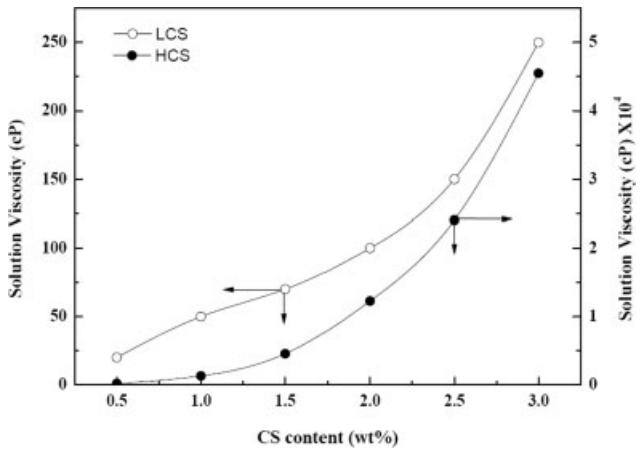

Figure 2 Viscosity of chitosan (CS)/PVA solutions with various CS concentrations.

ultraviolet (UV)–visible spectrometer (Shimadzu, UV-1700, Kyoto, Japan), and the antibacterial activity was evaluated quantitatively with the following equation:

$$\text{Antibacterial activity} = \frac{A - B}{A} \times 100\ (\%) \qquad (3)$$

where A and B are the numbers of surviving cells in the control and test samples, respectively.

RESULTS AND DISCUSSION

Viscosity

Relative viscosity is an important factor that affects the fiber diameter and the web morphology of electrospun fibers. First, chitosan solutions with different viscosities were used to determine the optimum intrinsic viscosity in the electrospinning process. In this study, the electrospinning solution of chitosan was mixed with a small amount of PVA, which was used as an increasing agent of viscosity to improve the fiber-forming properties. PVA increased the viscosity of the chitosan solution, which resulted from molecular interactions between chitosan and the hydrophilic hydroxyl ion of PVA. The reason why the viscosity with high-concentrated chitosan was not measured was that it was impossible for a chitosan solution with a concentration higher than 2.0 wt % not to be electrospun.

Figure 2 shows the relationship between the viscosity and the concentration of chitosan. The viscosity of chitosan with low intrinsic viscosity gradually increased from 0.5 to 2.0 wt %. The viscosity of the chitosan solution with high intrinsic viscosity increased with chitosan concentration and particularly showed a significant change in its viscosity at concentrations higher than 1.5 wt %, which resulted from the increasing bonding strength between the side chains of the chitosan with increasing chitosan concentration, which restricted its molecular movement. The diameter of the electrospun fibers was also affected by the concentration of chitosan. The number of beads in the electrospun fibers made the chitosan concentration higher than 7 wt %, which seemed to be the maximum concentration for forming nanofibers with uniform fiber diameter.

Electrospinning of the chitosan nanofibers

Figure 3 shows the relationship between the yield of the electrospun chitosan nanofibers and the concentration of chitosan. The chitosan nanofibers were obtained under conditions of a 1 mL/h flow rate, a 15 kV voltage, and a 10 cm TCD. The production yields of the LCS/PVA and HCS/PVA solution were 80 and 85%, respectively. The high production yield of HCS/PVA was higher than that of LCS/PVA because the increase in elongational tension of HCS/PVA resulted in the easy formation of fibers under the condition of a high electric field. By contrast, the decrease in the viscosity of chitosan provided a decrease in intramolecular forces among the chitosan molecules, which gave rise to a low production yield of nanofibers in the electrospinning process. These results indicate that the precise control of the viscosity of chitosan critically affects on the production yield of electrospun nanofibers.

Water uptake

The water content was very important in the electrospun fibers, which supported the ionization of functional groups in the electrospun fibers for the adsorption reaction. Figure 4 shows the water uptake of the chitosan nanofibers with the various chitosan ratios in the chitosan/PVA solutions. As shown in Figure 4, the water uptakes for the HCS and LCS nanofibers were 430 and 390%,

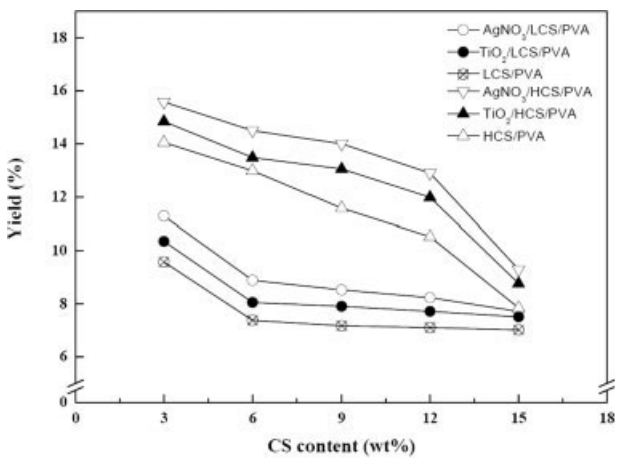

Figure 3 Effect of the chitosan (CS) concentration on the production yield of the CS/PVA nanofibers.

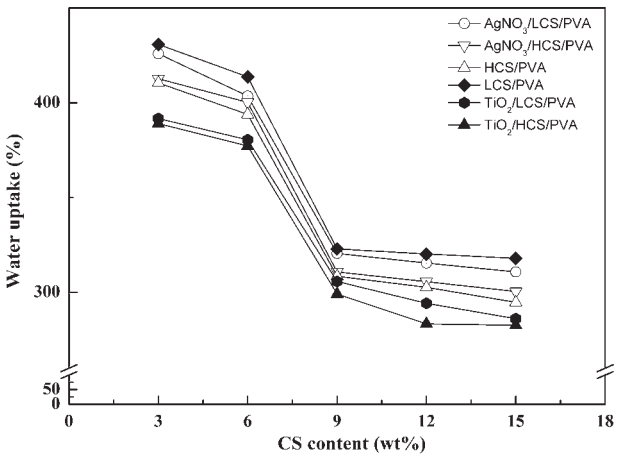

Figure 4 Effect of the chitosan (CS) concentration on the water uptake.

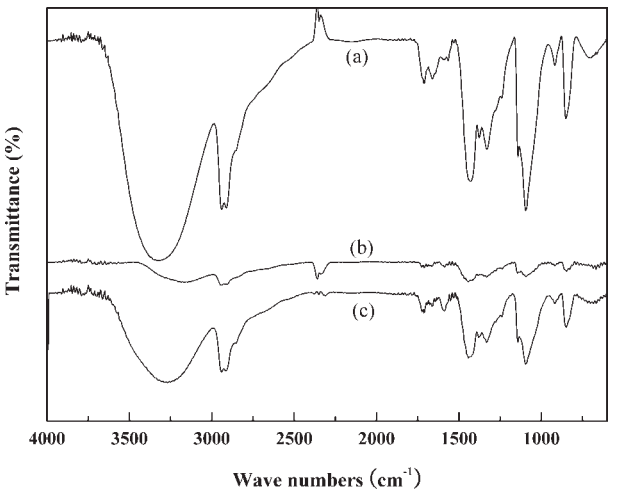

Figure 5 FTIR spectra of the electrospun chitosan nanofibers: (a) chitosan, (b) PVA, and (c) chitosan/PVA.

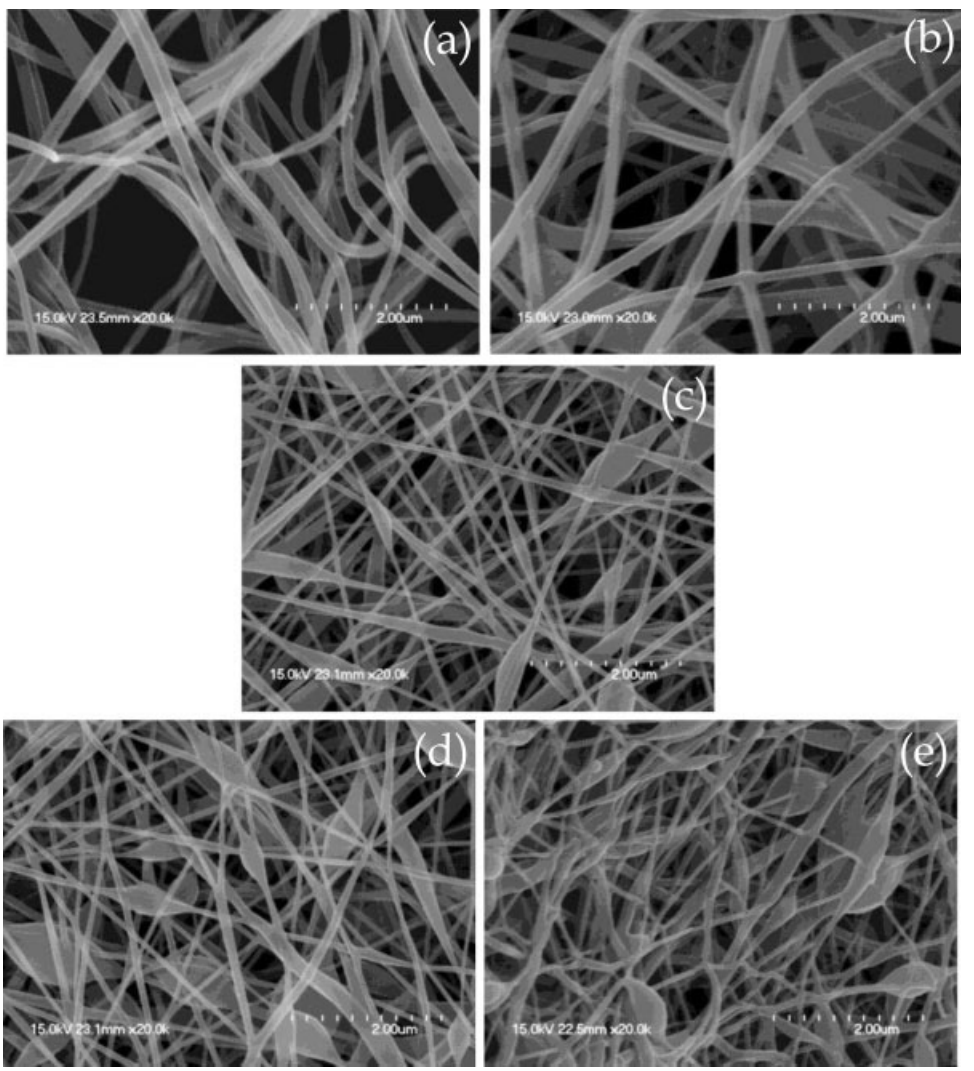

Figure 6 SEM photographs of the LCS/PVA nanofibers with LCS/PVA ratios of (a) 3 : 97, (b) 6 : 94, (c) 9 : 91, (d) 12 : 88, and (e) 15 : 85 wt %.

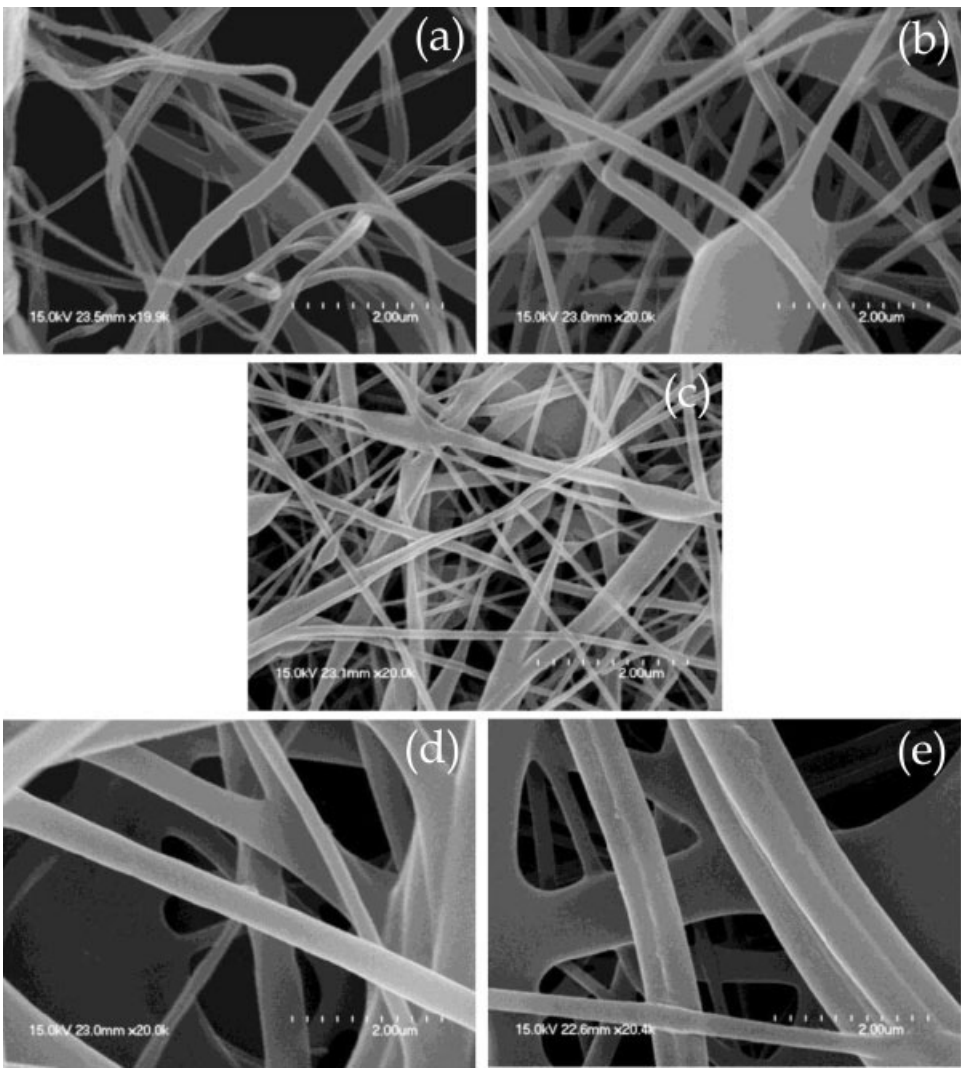

Figure 7 SEM photographs of the HCS/PVA nanofibers with HCS/PVA ratios of (a) 3 : 97, (b) 6 : 94, (c) 9 : 91, (d) 12 : 88, and (e) 15 : 85 wt %.

respectively, and tended to decrease with increasing chitosan concentration in the electrospinning solution. The latter had a higher water uptake than the former at concentrations higher than 15 wt %.

According to a previous study,[18] it seemed that with a high water uptake and low chitosan concentration, a high concentration of hydrophilic PVA in the electrospinning solution made it easy to form a polymeric hydrogel in the solution, which reduced the coagulant forces between molecules and then allowed easy swelling. Above 12 wt %, the intermolecular forces between the chitosan side chains and amine groups increased and affected the molecular structure and mobility, which led to a decrease in the swelling of nanofibers.

FTIR spectroscopy

The FTIR spectra of the electrospun nanofiber of the chitosan proved the existence of the PVA, chitosan, and chitosan/PVA nanofibers. The FTIR spectra showed the characteristic absorption bands for O—H and NH at 3450 cm^{-1}, C—H at 2907 cm^{-1}, CH_3 at 1383 cm^{-1}, and C—O at 1141 and 1085 cm^{-1}, which supported the configuration of PVA, as shown in Figure 5(a).[19]

Figure 5(b,c) show the absorption bands of the FTIR spectra for O—H (3450 cm^{-1}), N—H (3360 cm^{-1}), and N—H (1588 cm^{-1}) and the saccharine characteristic peak (1150 cm^{-1}–898 cm^{-1}), which confirm the structure of the chitosan/PVA nanofibers with hydrogen bonding.[20]

Surface morphology of the nanofiber

The electrospinning solution was electrospun with various changing parameters, such as the ratio of chitosan, PVA, antibacterial agents, flow rate, voltage, and TCD, to obtain optimized conditions for electrospinning the chitosan nanofibers. The effect of

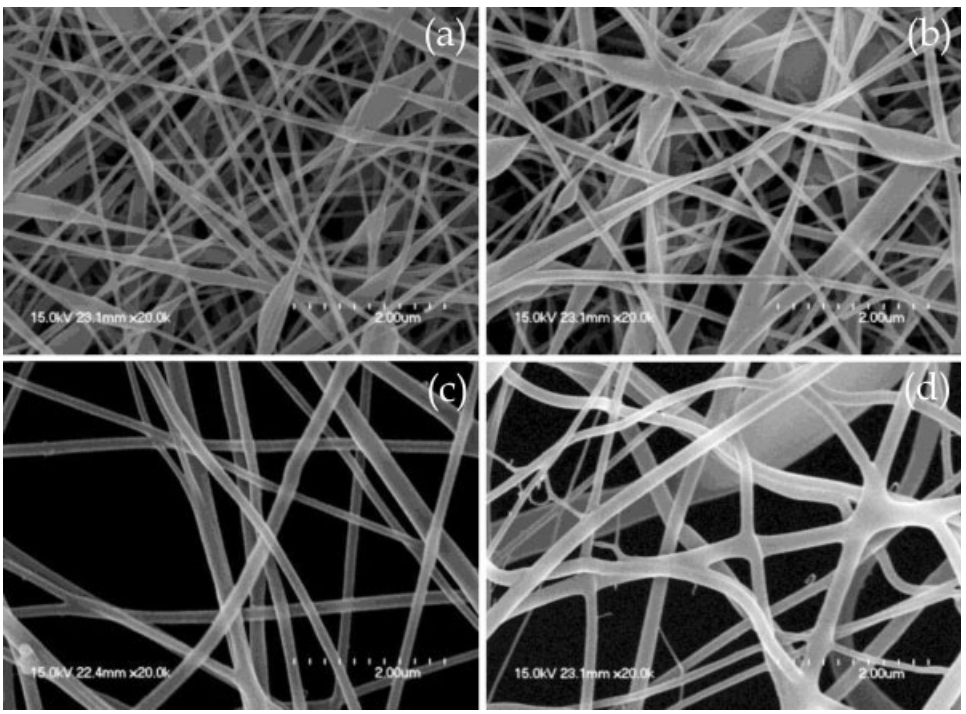

Figure 8 SEM photographs of the chitosan/PVA (30 : 70) nanofibers with $AgNO_3$ and TiO_2 nanofibers: (a) $AgNO_3$/LCS/PVA, (b) TiO_2/LCS/PVA, (c) $AgNO_3$/HCS/PVA, and (d) TiO_2/HCS/PVA.

these parameters on the electrospun nanofibers was analyzed by SEM.

Figure 6 shows the images of the electrospun nanofibers from the LCS/PVA solutions with different ratios. Uniform nanofibers were obtained at a ratio range of LCS of 3–15, and at higher LCS ratios, the formation of the bead gradually increased, and the fiber diameter became thicker to reduce the productivity of the nanofibers.

On the other hand, the SEM images of the nanofibers obtained from the HCS/PVA solution are shown in Figure 7. For all of the solution ratios, the electrospun nanofibers had an uneven fiber diameter, in particular, in the film with a HCS ratio higher than 30 wt %. The elongation force is a function of the viscosity of the electrospinning solution. Therefore, with the HCS solution, it was more difficult to electrospin the nanofibers. The viscosity of the LCS solution was lower than the surface tension, which provided efficient electrospinning of the chitosan solution.[21,22] Therefore, the HCS concentration was considered to be optimum for electrospinning below the ratio of 9 : 91.

Figure 8 shows the SEM image of the chitosan/PVA nanofibers with $AgNO_3$ and TiO_2 in range 0.01–0.04 wt %. As shown in Figure 8(a,b), the fiber diameters of the $AgNO_3$/LCS/PVA nanofibers were smaller and more uniform than those of the nanofibers having TiO_2 because the dispersion forces of $AgNO_3$ were higher than those of TiO_2. Although $AgNO_3$ in the chitosan solution dissociated to Ag^+ and NO_3^-, which tended to react with chitosan, TiO_2 was dispersed well as nanoparticles in the solution because it was not dissipated and did not form electrostatic attraction forces. The fiber diameter of the $AgNO_3$/HCS/PVA nanofibers showed a similar trend, as illustrated in Figure 8(c,d). Under the same electrospinning conditions, LCS nanofibers with uniform diameter were obtained, in contrast to the HCS nanofibers. This result was consistent with the explanation of the effect of the viscosity. The formation of a fine diameter in $AgNO_3$-added nanofibers seemed to occur because Ag accumulated charges in the solution and affected the charge density of the solution when it was electrospun.

Table II shows the fiber diameter with various parameters in the electrospinning conditions. The increase of applied voltage tended to decrease the fiber diameter, and chitosan nanofibers having uniform fiber diameters were obtained at 18 kV. The diameter also increased with electrospinning rate. At 7.5 cm TCD, chitosan/PVA nanofibers were

TABLE II
AFD of the Chitosan/PVA Nanofibers with $AgNO_3$ and TiO_2

Flow rate effect	Level (mL/h)	0.5	1.0	1.5	2.0
	AFD (nm)	275	295	342	260
Voltage effect	Level (kV)	9	12	15	18
	AFD (nm)	335	320	295	280
TCD effect	Level (cm)	7.5	10.0	12.5	15.0
	AFD (nm)	264	295	331	445

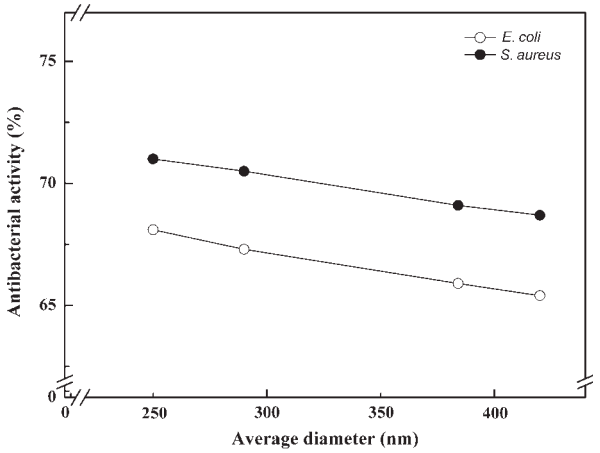

Figure 9 Effect of the average diameter of the nanofibers on the antibacterial activity.

produced with 264 nm average diameters; the diameter tended to increase with increasing TCD. This was due to weak dissipative forces at long TCDs.[21–23]

Antibacterial activity

Figure 9 shows that the antibacterial activity of the chitosan nanofibers of LCS/PVA was higher than that of HCS/PVA. The antibacterial activity of chitosan nanofiber depended on the functional groups of chitosan. The viscosity influenced only the production of nanofibers in the electrospinning process. The similar diameter of chitosan nanofibers provided the same exposed surface area (Fig. 9), which did not produce different antibacterial activities. All of the nanofibers made in this study had an average fiber diameter (AFD) in range 270–360 nm, and the effective surface area did not change very much. Therefore, the fiber diameter of the nanofibers had little effect on the antibacterial activity. Figure 10 clearly shows the effect of the concentration of chitosan on antibacterial activity. The high concentration of chitosan provided an increase in antibacterial activity. However, as shown in Figure 9, there was no critical effect of viscosity on the antibacterial activity.

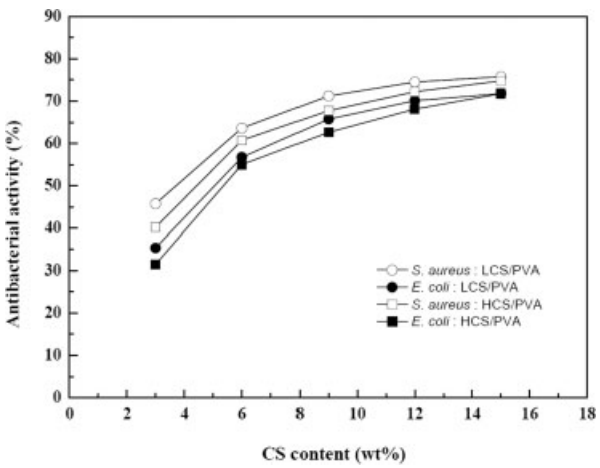

Figure 10 Effect of the chitosan (CS) concentration on the antibacterial activity.

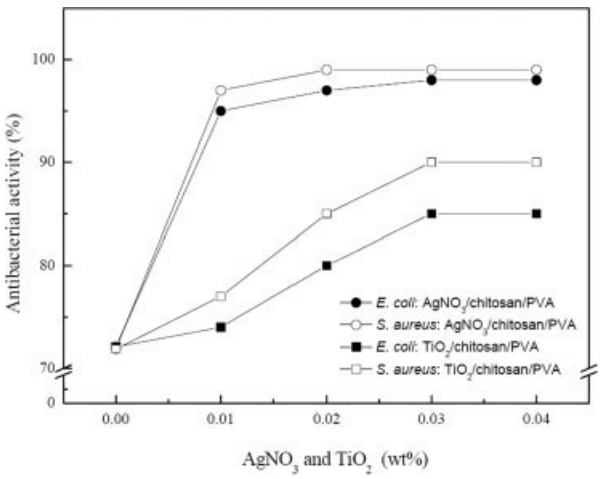

Figure 11 Effect of the $AgNO_3$ and TiO_2 concentrations on the antibacterial activity.

Figure 11 shows the antibacterial activity of the nanofibers with various concentrations of $AgNO_3$ and TiO_2. The antibacterial activity was increased with added $AgNO_3$ concentration and reached a maximum value at 0.04 wt % $AgNO_3$, with a killing rate of 99% for *S. aureus* and 98% for *E. coli*. Meanwhile, the TiO_2/HCS/PVA nanofibers maximum antibacterial activity at TiO_2 concentrations higher than 0.03 wt %, with 90% for *S. aureus* and 85% for *E. coli*. These experiments indicated that the chitosan/PVA/$AgNO_3$ nanofibers prevented bacteria from growing more effectively. The antibacterial mechanism of Ag on the bacteria differed from that of TiO_2. The $AgNO_3$ contained in the nanofibers was released rapidly and steadily as a type of Ag^+ ion, which reacted with the bacteria.[24] In general, TiO_2 can be improved to produce radicals under the conditions of the irradiation of UV light. The TiO_2/chitosan/PVA nanofibers, however, were not illuminated under UV light during the antibacterial experiment and eventually showed lower antibacterial activity than the $AgNO_3$-added nanofibers. As expected, the nanofibers with embedded antibacterial agents had higher activity against gram-negative and gram-positive bacteria. These result support the conclusion that the addition of $AgNO_3$ and TiO_2 is a proper and effective method for the development of chitosan/PVA nanofibers with good antibacterial function.

CONCLUSIONS

A chitosan/PVA solution dissolved in formic acid was electrospun to produce chitosan/PVA nanofibers

with $AgNO_3$ and TiO_2. We found that the optimized conditions for the chitosan/PVA nanofibers were a 0.5 mL/h flow rate, an 18 kV voltage, and a 7.5 cm TCD with a diameter in the range 270–360 nm. The water uptake on the nanofibers depended on the concentration of the chitosan. From the experimental results through the shake flask method, the chitosan/PVA nanofibers with $AgNO_3$ and TiO_2 exhibited better antibacterial activity on *E. coli* and *S. aureus* than those without antibacterial reagents, and the diameter of the nanofibers affected the antibacterial activity. The chitosan/PVA nanofibers showed possibility as effective antibacterial fibers through the addition of the functional antibacterial reagents.

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