Electrospinning of Antibacterial Poly(vinylidene fluoride) Nanofibers Containing Silver Nanoparticles

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ABSTRACT: Poly(vinylidene fluoride) (PVDF) nanofibrous mats containing silver nanoparticles were prepared by electrospinning. The diameter of the nanofibers ranged between 100 and 300 nm, as revealed by scanning electron microscopy. The silver nanoparticles were dispersed, but some aggregation was observed with transmission electron microscopy. The content of silver nanoparticles incorporated into the PVDF nanofibrous mats was determined by inductively coupled plasma and X-ray photoelectron spectroscopy. The antibacterial activities of the samples were evaluated with the colony-counting method against *Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative) bacteria. The results indicate that the PVDF nanofibrous mats containing silver nanoparticles showed good antibacterial activity compared to the PVDF nanofiber control. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 668–672, 2010

Key words: fibers; nanoparticles

INTRODUCTION

Poly(vinylidene fluoride) (PVDF) shows excellent mechanical properties and resistance to severe environmental stress and good chemical resistance and has been processed into a separation filter.[1] However, because of bacterial growth and congestion on the surface of the filter media, the pore size decreases gradually.[2] Although the incorporation of biocides can prevent microbial colonization, the applications are limited because of short-term killing efficacy. In addition, the release of biocides has the potential to increase bacterial resistance to biocides. Therefore, filter materials with bacterial-growth-inhibition properties are desired for scientific and industrial applications. Small fibers in the submicrometer range, in comparison with larger ones, are well known to provide better filter efficiencies at the same pressure drops in the interception and inertial impaction regimes.

Electrospinning is known to be a novel and efficient fabrication tool for preparing fibrous polymer membranes with fiber diameters ranging from several micrometers down to tens of nanometers.[3] Electrospun fibers show extraordinary properties, such as dramatically increased surface-to-volume ratios, excellent mechanical strength, and highly open porous structures. Previously, Zhao et al.[4] studied the effects of the solvent ratio, polymer concentration, and capillary–collector distance on PVDF electrospinning. In recent years, nanofibrous PVDF has been reported to be used as a polymer electrolyte.[5–7] Manesh et al.[8] electrospun PVDF/poly(aminophenylboronic acid) composite nanofibrous membranes used as novel glucose sensors, which displayed excellent linear responses to the detection of glucose and possessed better reproducibility toward glucose detection than that of poly(aminophenylboronic acid). PVDF composites with carbon nanotubes can improve the mechanical properties of the matrix polymer.[9] Silver nanoparticles exhibit antibacterial properties and have been incorporated into polymeric materials to achieve antibacterial applications, such as wound dressing,[10] water purification,[11] and household usage.[12] Alt et al.[13] reported poly(methyl methacrylate) (PMMA) bone cement loaded with 5–50-nm silver particles that was free of *in vitro* cytotoxicity and showed a high effectiveness against multiresistant bacteria. Wang and coworkers[14,15] incorporated silver nanoparticles into polyacrylonitrile and silica nanofibers, which exhibited good conductive and catalytic properties. Duan et al.[16] and Xu et al.[17] introduced silver nanoparticles into nanofibrous polycaprolactone and poly(L-lactide), respectively, which were antimicrobial, biodegradable, and biocompatible. To this point, silver nanoparticles

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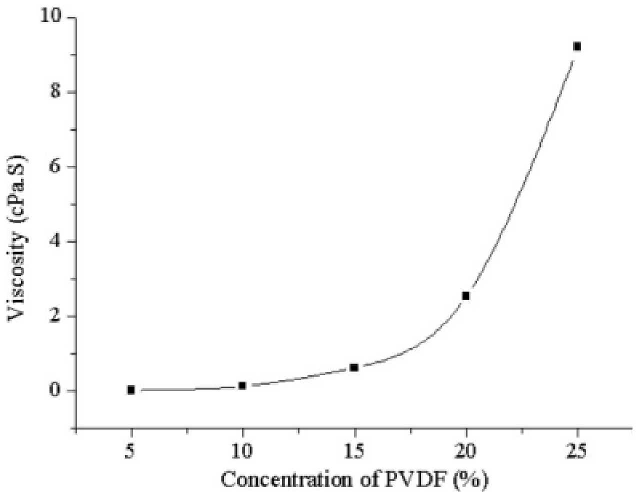

Figure 1 Solution viscosity versus PVDF concentrations.

have been incorporated into nanofibrous polymers, including polyvinylpyrrolidone,[18–21] poly(vinyl alcohol),[22,23] polyacrylonitrile,[14,24,25] polyimide,[26] and cellulose acetate.[27]

In this study, silver nanoparticles were first suspended in the PVDF solution and then electrospun to produce antibacterial PVDF mats. The nanofibrous mats were characterized with scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), and inductively coupled plasma (ICP). The antibacterial activity of the mats was evaluated with *Staphylococcus aureus* and *Klebsiella pneumoniae*.

EXPERIMENTAL

Materials

PVDF (weight-average molecular weight = 275,000) and *N*,*N*-dimethylacetamide (DMAc) were purchased from Sigma-Aldrich (USA) and were used without further purification. The silver nanoparticle solution in methanol was donated by Hunion Co. (Seoul, Korea).

Electrospinning of PVDF

A transparent polymer solution for electrospinning was obtained by the dissolution of PVDF in DMAc with sufficient stirring at room temperature. The solution was delivered to a metal needle (18 G) connected to a high-voltage power supply (Chungpa EMT, Seoul, South Korea). Upon the application of high voltage, a jet of fluid was ejected from the needle. As the jet accelerated toward a grounded collector, the solvent evaporated, and a charged polymer fiber was deposited on the collector in the form of a nanofiber mat. Several parameters for electrospinning, including solution concentration, voltage, flow rate, and distance between tip and collector, were optimized. The morphology of the as-spun mat was examined with field emission scanning electron microscopy (FE-

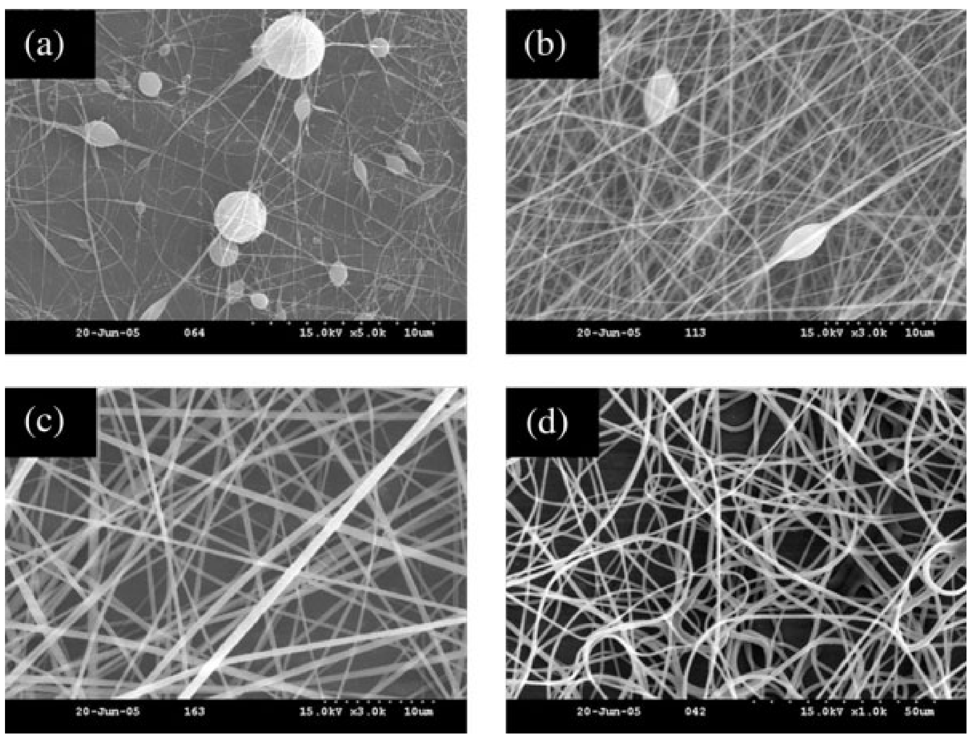

Figure 2 SEM micrographs of electrospun PVDF versus concentration at 20 kV, 20 cm, and 1 mL/h: (a) 10, (b) 15, (c) 20, and (d) 25%.

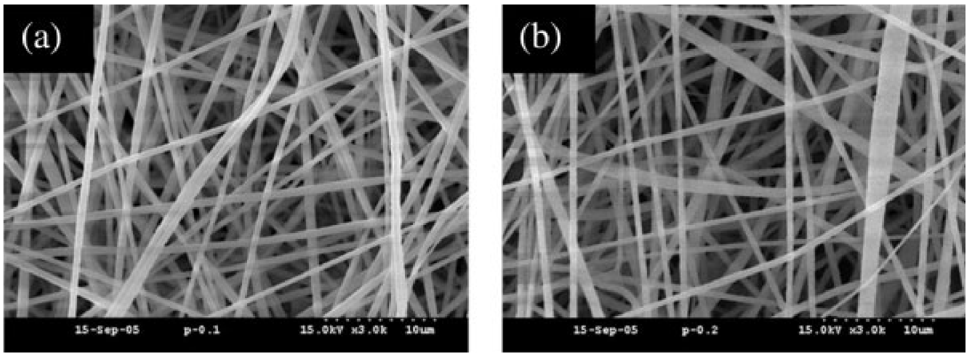

Figure 3 SEM micrographs of electrospun PVDF mats containing silver nanoparticles: (a) 280 and (b) 676 ppm silver content.

SEM). Under optimized conditions, 280 and 676 ppm calculated amounts of silver solution were suspended in PVDF/DMAc solutions for electrospinning.

Surface characterization method

The viscosity of the PVDF solution was measured with a Brookfield viscometer (RVTD model, USA). The temperature was maintained at 25 ± 2°C during the measurements with a thermostatically controlled tank. The morphology of the nanofibrous mats was observed with FE-SEM (Hitachi S-4300, Japan). The silver content was analyzed by an ICP spectrometer (Themo Jarrell Ash Co., IRIS/AP, USA) and an XPS instrument (ESCA LAB VG Microtech, Mt500/1etc, East Grin, United Kingdom) equipped with Mg Kα at 1253.6 eV and 150 W of power at the anode. The shape and distribution of silver particles in the PVDF nanofibers was observed with high-resolution TEM (Hitachi H7600 TEM).

Antibacterial assessment[28]

S. aureus and *K. pneumoniae* were cultivated in a nutrient broth for 24 h in a CO_2 incubator. The nanofibrous mats were sterilized in an autoclave and cut to sizes of 1 × 1 cm^2. The diluted bacterial suspension was cultured in a vial containing 0.4 g of the samples. The vials were incubated at 37°C for 18 h. The bacteria collected from each vial were plated onto agar medium. After incubation at 37°C for 24 h, the resulting bacterial colonies in the plates were counted visually. The percentage of bacterial growth inhibition was calculated with the difference between the numbers of colonies from the bacteria with samples and those from bacteria in the vials as a control. All experiments were performed in triplicate, and the quantitative value was expressed as the average plus or minus the standard deviation.

RESULTS AND DISCUSSION

Electrospinning

The viscosity of the PVDF solution in DMAc was first measured to determine the appropriate concentration for electrospinning, and the results are shown in Figure 1. The viscosity of the PVDF solution increased very slowly up to 15 wt % and, thereafter, increased rapidly. The polymer concentration is one of the most significant parameters for controlling the electrospun fiber morphology. Figure 2 shows SEM micrographs of the electrospun fibers versus solution concentration. For the low polymer concentration of 10%, a large number of beads and bead fibers appeared [Fig. 2(a)]. The beads disappeared at a concentration of 20%, and the diameter of the obtained fibers was 600 ± 176 nm [Fig. 2(c)]. When the solution concentration increased to 25%, the electrospun fiber could not spread straight because of its excessive viscosity [Fig. 2(d)]. Finally, the parameters for electrospinning were fixed as follows: 20 wt % (concentration), 20 kV (voltage), 20 cm (distance between the tip and the receiver), and 1 mL/h (flow rate).

A calculated amount of silver solution was added to achieve final silver concentrations of 280 and

TABLE I
Silver Content of the Electrospun PVDF/Silver Nanofibers as Determined by ICP Spectrometry

	Ag content	
Sample	Prepared	Found
PVDF-S1	280 ppm	310 ppm
PVDF-S2	676 ppm	730 ppm

TABLE II
Chemical Composition of the Electrospun PVDF/Silver Nanofibers Calculated from the XPS Spectra

	Atomic percentage		
Sample	C	F	Ag
PVDF-S0	18.8	81.2	0
PVDF-S1	16.7	82.3	1.0
PVDF-S2	15.3	83.4	1.2

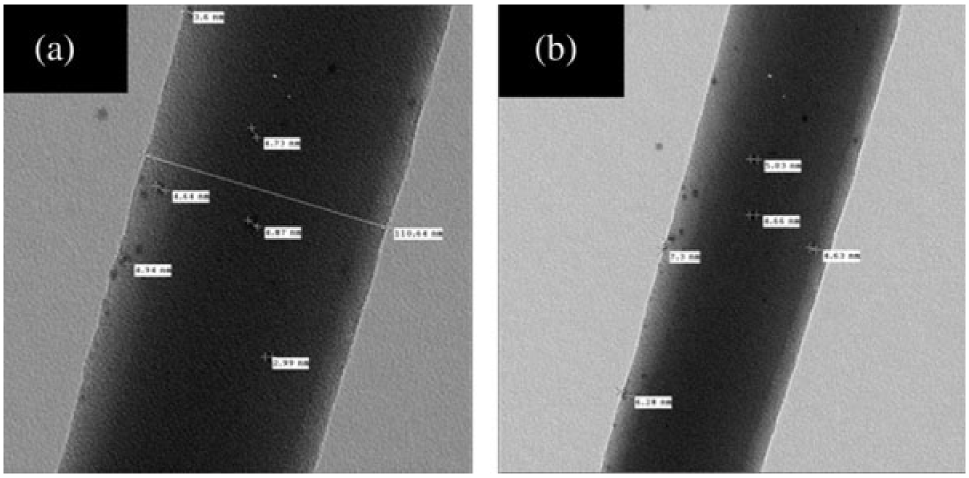

Figure 4 TEM images of silver nanoparticles distributed in the PVDF nanofibers: (a) PVDF-S1 and 280 ppm silver content and (b) PVDF-S2 and 676 ppm silver content.

676 ppm, respectively. Figure 3(a,b) shows the SEM images of PVDF fibers containing silver nanoparticles. Compared with the blank PVDF fiber, these fibers were quite smooth and became finer with an average diameter of 300 nm. The addition of silver increased the conductivity of the polymer solution, so the fiber diameter decreased with increasing silver concentration. This was because a solution with a higher electrical conductivity would have caused a higher elongation of a jet along its axis and, thus, electrospinning fibers with smaller diameters.[29] The fiber would have been covered with some silver stains and become rough when a large amount of silver solution was adopted.

Silver content analysis

The silver content was analyzed by ICP spectrometry and XPS. The silver contents prepared and found by ICP spectrometry are listed in Table I. The agreement of the obtained results with the prepared values was very high. Table II shows the elemental compositions calculated from the XPS spectra. The silver contents were 1.0 and 1.2%; this indicates that the amount of silver on the surface of the fibers was very low.

Silver nanoparticle distribution

A piece of Ag/PVDF nanofibrous mat was stuck on a copper grid to observe the shape and distribution of silver particles in the PVDF nanofibers by high-resolution TEM. Figure 4 shows the silver nanoparticles that existed both on the surface and inside the nanofiber. However, a small part of the silver nanoparticles was distributed with some extent of aggregation. The average diameter of the silver nanoparticles was 5.1 nm. The silver particles on the surface played a more important role in the antibacterial mechanism.

Antibacterial activity

We evaluated the antibacterial activity of the nanofibrous mats by counting the colonies that formed on the plates, and the growth inhibition rate was calculated from the following equation: Growth inhibition rate (%) $= (B - A)/B \times 100$, where A and B are the number of colonies from the bacteria in the vial with the samples and in the blank vial, respectively. Figure 5 and 6 show the antibacterial assessment results of the PVDF mats containing silver nanoparticles and blank mats on the growth inhibition rate of *S. aureus* and *K. pneumoniae*. Growth inhibition appeared on both of them, independent of the bacterial strain. As expected, the antibacterial ability was enhanced with the higher silver content. When the mats were immersed, the silver on the surface released easily, and the silver inside diffused to the

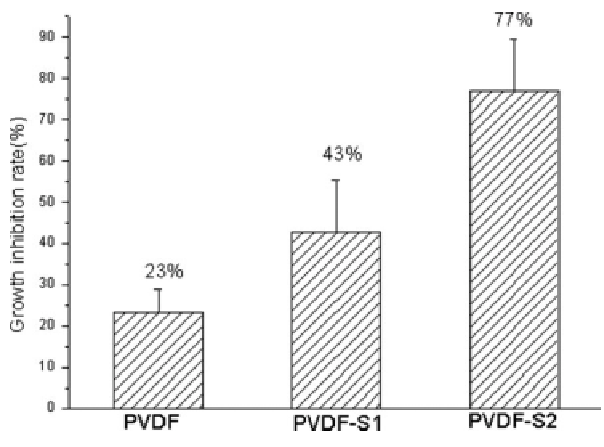

Figure 5 Growth inhibition rates of the PVDF nanofibrous mats against *S. aureus* with different amounts of silver (PVDF-S1, 280 ppm, and PVDF-S2, 676 ppm).

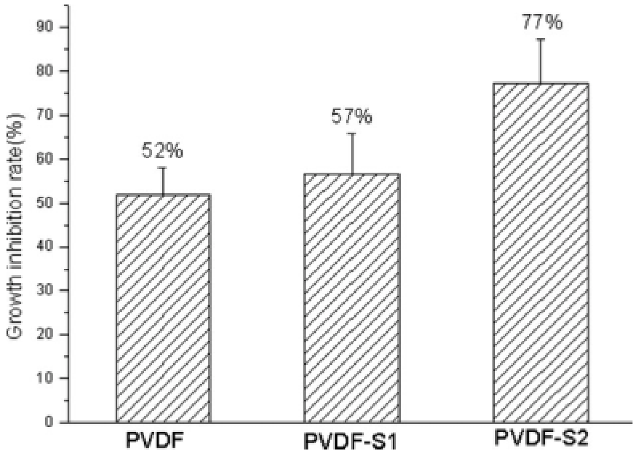

Figure 6 Growth inhibition rates of PVDF nanofibrous mats versus *K. pneumonia* with different amounts of silver.

outside slowly. It was these reasons that led to a long-term antibacterial effect. One of the major bactericidal actions of the silver ion is caused by its interaction with the ribosome and subsequent suppression in the expression of enzymes and proteins essential to adenosine triphosphate (ATP) production.[30] The blank PVDF in this study had some antibacterial properties. PVDF tubes were explored to inhibit biofilm formation in dental unit waterlines.[31] The intermolecular force between the bacterial cells and solid surface became weaker because of its low surface free energy, which made it less possible for the bacteria to adhere. Interestingly, the Gram-positive bacteria (*S. aureus*) were the most affected compared to the Gram-negative bacteria (*K. pneumoniae*). This difference may have been due to dissimilarities in the cell wall materials of the two types of bacteria. The Gram-positive cell walls were principally composed of peptidoglycan (90%), a macromolecule composed of amino acids and sugar, which has the ability to form more than 20 layers in the cell wall. In addition, the *Bacillus* species have the ability to form endospores, which are highly resistant to a wide range of biocidal agents. The Gram-negative bacteria, however, are low in peptidoglycan but high in lipids.[32]

CONCLUSIONS

PVDF nanofibrous mats containing silver nanoparticles were prepared by electrospinning. The silver nanoparticles distributed with some extent of aggregation. The PVDF nanofibrous mats containing silver nanoparticles showed good antibacterial activity compared to the blank PVDF nanofibrous mat, especially for Gram-positive bacteria (*S. aureus*). The obtained PVDF/silver nanofiber mats may find practical applications, such as in water filters, wound dressings, or antiadhesion membranes.

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