Antimicrobial Fiber Products Treated with Silica Hybrid Ag Nanoparticles

Hwa-Jung Kim, Hae-Jin Park[1], Seong-Ho Choi[2] and Hae-Jun Park*

Radiation Research Division for Biotechnology Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-815, Korea
[1]Biodreams Co. Ltd. Bio Venture Center 204 Korea Research Institute Bioscience and Biotechnology, Yuseong, Daejon 305-333, Korea
[2]Department of Chemistry, Hannam University, Daejeon 305-811, Korea

Abstract - Silica hybrid silver nanoparticles showing the strong antimicrobial activity, in which nano-silver is bound to silica molecules, has been synthesized using γ-irradiation at room temperature. The present study relates to an antimicrobial composition for coating fiber products comprising silica hybrid silver nanoparticles. In this study, we describe antimicrobial fiber products coated with the silica hybrid silver nanoparticles and a method of antimicrobially treating fiber products by coating the fiber products with the silica hybrid silver nanoparticles. The antimicrobial fiber products exhibited excellent antimicrobial effects. In detailed practice, when the present composition comprising nanosized silica-silver was applied to a cloth (fabric) in a concentration of 6.4 mg yard^{-1}, the viable cell number decreased to less than 10 cells before and after laundering, resulting in a reduction of 99.9% or greater in the viable cell number. The present composition displays long-lasting potent disinfecting effects on bacteria. Also, we investigated the toxicity of silica hybrid silver nanoparticles in rats. The skin of rats was treated with a 30 ppm nanoparticles solution (2 ml Kg^{-1}) for 8 days. No toxicity was detected in the treatment. These results suggest that the fiber products coated with the silica hybrid silver nanoparticles can be used to inhibit the growth of various microorganisms.

Key words : Silver nanoparticle, Nanosized silica hybrid, γ-irradiation, Antimicrobial fiber

INTRODUCTION

The antimicrobial fiber products are generally manufactured mainly by coating the surface of fiber products with various antimicrobial substances. A conventional method of coating fiber products with antimicrobial substances mainly employs a colorless water-soluble liquid, N, N-Dimethylformamide (DMF) or methyl ethyl ketone (MEK), as a solvent (Wang *et al.* 2009; Raghuvanshi *et al.* 2009). However, DMF, used as a solvent, irritates the respiratory tract, skin, eyes, and the like. Also, DMF is very well absorbed into the body through the skin and injures the liver due to its toxicity (Wang *et al.* 2009). MEK is also a chemical compound very harmful to the human body (Raghuvanshi *et al.* 2009). MEK also irritates the respiratory tract, skin, eyes, and the like. When inhaled, DMF and MEK cause vomiting, difficulty in breathing, headache, drowsiness and dizziness, and in severe cases, causes central nervous system disorders.

The use of these harmful chemical compounds for coating antimicrobial substances generates a great quantity of environmentally harmful substances during the manufacturing of antimicrobial fibers. In addition, antimicrobial fiber pro-

* Corresponding author: Hae-Jun Park, Tel. +82-63-570-3190, Fax. +82-63-570-3149, E-mail. hjpark@kaeri.re.kr

ducts prepared using the solvents become very slippery when it rains or when the fiber products become wet, due to characteristics of the solvents, and have the risk of growing harmful fungi capable of generating offensive odors and causing dermatitis. The solvents have a further problem in that long-term use hardens fiber products.

In order to solve the aforementioned problems, fiber products having antimicrobial effects are manufactured using silver (Ag) particles. Ag, which is known as a strong bactericidal agent, destroys unicellular microorganisms through its antimicrobial activity against enzymes performing metabolic functions in microbes (Kim *et al.* 1998; O'Neill *et al.* 2003; Thomas *et al.* 2003; Mallick *et al.* 2004; Sondi *et al.* 2004; Lee *et al.* 2005; Oh *et al.* 2006; Gaidau *et al.* 2009; Kim *et al.* 2009; Min *et al.* 2009; Li *et al.* 2010). When Ag exists in an ionic state, it has good antimicrobial activity. However, Ag is unstable due to its high reactivity and is easily oxidized or reduced to metal according to the surrounding atmosphere, thereby spontaneously changing in color or causing other materials to be changed in color (Park *et al.* 2006). These phenomena lead to a reduction in the duration of the antimicrobial action of Ag. When present in metal or oxidized form, silver is stable in the environment but must be used in relatively large amounts due to its low antimicrobial activity. Ag, having the advantages and drawbacks as noted above, is spotlighted in the form of nanoparticles for antimicrobial fiber products (Lee *et al.* 2005). Nanoparticles are synthesized by physical methods, such as mechanical grinding and wet reduction. Of the physical methods, the electrolysis technique requires high production costs, has difficulty producing nanoparticels in a large scale, and has difficulty controlling the size of formed particles. In contrast, a method of preparing nanometer-sized particles by irradiation with radiation rays has the following advantages: it easily controls the size, shape and size distribution of particles; it can form nanoparticles at room temperature; and it provides a simple process and thus makes mass production possible with low costs (Oh *et al.* 2006; Park *et al.* 2007). In addition to the aforementioned methods, many efforts have been made to provide nano-silver applicable to a broad range of fields with purposes including anti-bacteria, cleaning and deodorization. Despite these efforts, there is still a need for the development of a simpler process for preparing cheaper and more stable nano-silver.

Based on this background, we prepared fiber treated with nanosized silica-silver particles (NSS), in which nano-silver is bound to silica molecules and a water-soluble polymer, and we further found that fabrics coated with such antimicrobial particles retain antimicrobial activity even after being laundered and dried.

MATERIALS AND METHODS

1. Chemicals

Silver nitrate ($AgNO_3$, 99.8%) and sodium silicate solution (Na_2SiO_3) were prepared analytical reagent grade. Isopropylalcohol (IPA, 99%) was purchased from Merk Co., polyvinylpyrrolidone (PVP, 99.9%) from Acros Co., and potato dextrose agar (PDA) from Difco Co. Fresh solutions were prepared daily for use. Distilled deionized water (DDW) with a resistance of 18.2 MΩ cm^{-1} was utilized for all the solutions that were used in the experiment.

2. Preparation of a nanosized silica hybride silver (NSS) complex

The NSS was obtained as follows. $AgNO_3$ (1.0 g), Na_2SiO_3 (1.0 g), PVP (1.0 g), and isopropyl alcohol (12 ml) were dissolved in DDW at a total volume of 200 ml. Oxygen was removed from the solution by bubbling with pure nitrogen for 30 min. The resultant solution was irradiated (30 kGy) with the use of a ^{60}Co γ-irradiator (150 TBq capacity; ACEL, Canada) at the Korea Atomic Energy Research Institute, for the preparation of NSS (Park *et al.* 2006; Park *et al.* 2007).

3. Preparation of antimicrobial fiber products

A fabric specimen was prepared by the above method. A polyester fabric (control specimen) to be coated with nanosized silica-silver was allowed to pass through a staining bath, a roller and a dryer to produce a test specimen. In the staining bath, the fabric was allowed to absorb about 100 ml $yard^{-1}$ (about 6.4 mg $yard^{-1}$) of the nanosized silica-silver (hereinafter, referred to as "NSA") prepared according to the method used for in a 2-fold dilution (hereinafter, referred to as "NSB") of the nanosized silica-silver with a levan solution. In order to remove water absorbed along with the nanosized silica-silver, the fabric was compressed using a roller, thus removing a part of water, then and was dried in a dryer

at 140°C for 15 min to completely remove the remaining water.

4. Determination of antimicrobial activity

It was carried out according to a test method for antimicrobial the activity of fabrics, (K0693)-2001, provided by Korean FITI Testing & Research Institute.

Staphylococcus aureus strain 209 (American Type Culture Collection No. 6538) and *Klebsiella pneumoniae* (American Type Culture Collection No. 4352) were streaked onto nutrient agar plates (prepared by dissolving 5 g of peptone and 3 g of beef extract in 1000 ml by heating, adjusting the resulting medium to pH 6.8±0.2 with 0.1 ml l^{-1} of sodium hydroxide and adding 15 g of agarose to the nutrient medium), and were cultured for 24 to 48 hrs at 37±1°C.

The two known bacteria, cultured as described above, were inoculated in 100-ml Erlenmeyer flasks containing 20 ml of a nutrient medium (prepared by dissolving 5 g of peptone and 3 g of beef extract in 1000 ml by heating and adjusting the resulting medium to pH 6.8±0.2 with 0.1 ml l^{-1} of sodium hydroxide), and were cultured for 24 to 48 hrs at 37±1°C with agitation. Thereafter, absorbance was measured using a spectrophotometer to determine cell density. Each culture was then adjusted to a density of $1\pm0.3\times10^5$ cells ml^{-1} with the 20-fold diluted nutrient medium precooled to 0°C, and was used as an inoculum in a fabric specimen.

5. Determination of antimicrobial stability

The control and test specimens, prepared using the above method, were placed into about 30 ml sterile glass containers each having a screw-type cap. Exactly 0.2 ml of the inoculum prepared above was inoculated in the containers while evenly placed on the control and test specimens. The inoculated control and test specimens were incubated at 37±1°C for 18 hrs.

Immediately after inoculation and at 18 hrs later, 20 ml of an neutralization solution precooled to 0°C (prepared by dissolving 5 g of sodium chloride and 2 g of a nonionic surfactant in 1000 ml of distilled water, aliquotting 20 ml of the solution into 100-ml Erlenmeyer flasks, and autoclaving the flasks in a high-pressure sterilizer under a steam pressure of 1055 g cm^{-3} at 120±2°C for 20 min) was added to the glass containers containing the inoculated control and test specimens. The flasks were vigorously shaken to release bacterial cells from the fabric specimens into the neutralization solution. The neutralization solution was then serially diluted in physiological saline by 10^0, 10^1, 10^2, 10^3 and 10^4 times. Exactly 1 ml of each dilution was collected, placed onto a petri dish, evenly mixed with 15 ml of nutrient agar medium at 45 to 46°C, and incubated at 37°C for 24 to 48 hrs. Thereafter, the viable cell number was calculated according to the following Equation 1.

$$M = Z \times r \times 20 \quad (1)$$

(M: viable cell number, Z: colony number, r: dilution time, 20: the amount of physiological saline used for release of bacterial cells)

On the other hand, to determine whether a fabric coated with nanosized silica-silver retained antimicrobial effects after laundering, the above fabric was laundered using an agitator-type automatic washing machine, and the viable cell number was measured. Laundering was carried out as follows. The agitator-type automatic washing machine was set to a cleaning cycle of 2 min, and filled with water at 40±3°C. The control and test specimens were placed into the washing machine along with a fabric for weight calibration, prescribed by the KS K 0465 method, to give a total weight of 0.9 kg. After 90 g of a commercial laundry detergent powder (Trade name: One-Scoop, LG Household & Health Care Co., Ltd.) was added to the washing machine, the washing machine was allowed to run. Rinsing was carried out using water at 30±3°C. The fabric specimens were then horizontally placed on a mosquito net and allowed to air-dry for 1 day. After washing and drying, the control and test specimens were inoculated with the inoculum prepared above, and the viable cell number was measured by a viable cell counting method. A reduction of viable cells in the fabric as treated above was determined according to Equation 2, below.

$$\text{Percentage reduction} = [(M_a - M_b)/M_a] \times 100 \quad (2)$$

(M_a: the number of viable cells in the control specimen after incubation for 18 hrs; M_b: the number of viable cells in test specimen after incubation for 18 hrs)

6. Acute dermal toxicity

The acute demal toxity of the nanosized silica-silver particles in rats were examined according to the OECD principle of good laboratrory practice. The naked skin of rats was

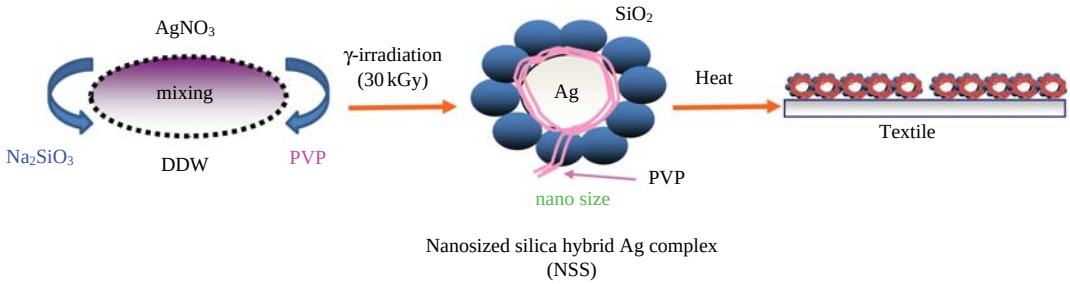

Fig. 1. Reaction procedures of NSS preparation by γ-irradiation.

treated with gauze (4 × 4 cm) sprayed with a 30 ppm nanoparticle solution (2 ml Kg^{-1}) for 24 hrs.

7. Instrumentation

The morphologie of NSS particles was then determined via field emission transmission electron microscopy (FE-TEM; HD-2300, Hitachi Ltd., Japan).

RESULTS AND DISCUSSION

1. A nanosized silica hybride silver (NSS) complex

Fig. 1 shows the reaction mechanism for the formation of NSS by γ-irradiation. The complex represents a structure in which NSS formed from an Ag ion, PVP, and Na_2SiO_3 is simultaneously polymerized and subjected to a reduction procedure for the dispersed nano-Ag particles within the PVP and SiO_2 molecules according to previous studies (Park *et al.* 2006; Park *et al.* 2007; Kim *et al.*; 2008; Kim *et al.* 2011).

The TEM images and EDX (Energy dispersive X-ray spectroscopy) analysis showed uniform spherical-type morphologies with an average size of 10 nm, and the presence of zero-valent Ag in the complex, as shown in Fig. 2. The nanosized silica-silver thus prepared is present study in a form in which nanoparticles are dissociated from each other at a colloidal state or assembled into loose spherical aggregates. Nano-silver particles in which nano-silver is coated with silica particles were conventionally developed.

2. Antimicrobial activity

In Table 1, the control specimen is a known fabric not having undergone antimicrobial treatment, which is employ-

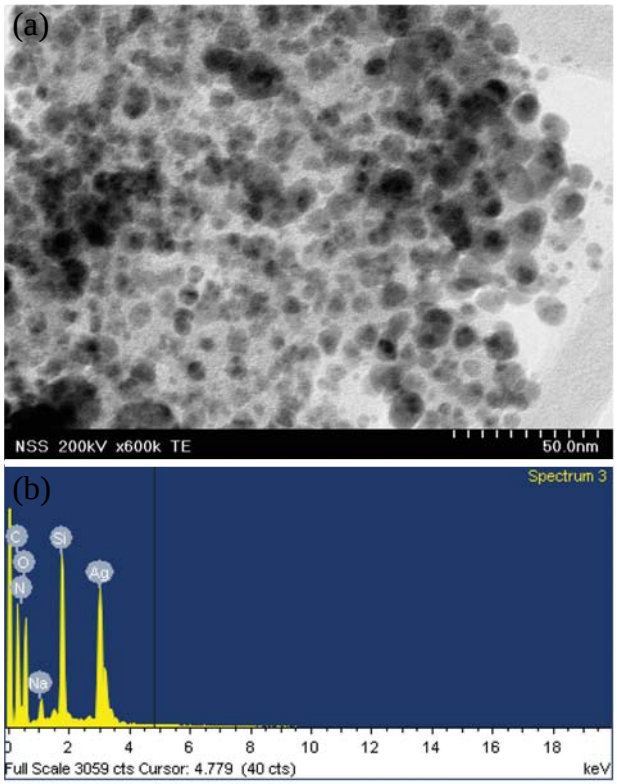

Fig. 2. FE-TEM images (a) and EDX data (b) of NSS.

ed in a test method for antimicrobial activity of fabrics provided by the Korean FITI Testing & Research Institute. The antimicrobial fiber products of the present investigation exhibited excellent antimicrobial effects. In detailed practice, when the present composition comprising nanosized silica-silver was applied to a cloth (fabric) in a concentration of 6.4 mg $yard^{-1}$, the viable cell number decreased to less than 10 cells before and after laundering, resulting in a reduction of 99.9% or greater in the viable cell number, as given in Table 1.

Our previous results suggested that the following action

Table 1. The antimicrobial activity of a fabric coated with nanosized silica-silver on the fabric treated with NSS

Fabric specimens	*Staphylococcus aureus* strain 209			*Klebsiella pneumoniae*		
	Initial cell number (cells ml^{-1})	Cell number after 18 hrs (cells ml^{-1})	Reduction (%)	Initial cell number (cells ml^{-1})	Cell number after 18 hrs (cells ml^{-1})	Reduction (%)
Control specimen	2.1×10^4	4.1×10^6	–	2.0×10^4	6.3×10^6	–
NSA	2.1×10^4	<10	99.9	2.0×10^4	<10	99.9
NSB	2.1×10^4	7.5×10^2	99.9	3.0×10^4	<10	99.9
NSA laundered once	2.1×10^4	<10	99.9	2.0×10^4	<10	99.9
NSB laundered once	2.1×10^4	<10	99.9	2.0×10^4	1.7×10^3	99.9

Table 2. The antimicrobial activity of the fabric laundered once against bacteria

Fabric specimens	*Staphylococcus aureus* strain 209			*Klebsiella pneumoniae*		
	Initial cell number (cells ml^{-1})	Cell number after 18 hrs (cells ml^{-1})	Initial cell number (cells ml^{-1})	Cell number after 18 hrs (cells ml^{-1})	Initial cell number (cells ml^{-1})	Cell number after 18 hrs (cells ml^{-1})
Control	2.1×10^4	4.9×10^6	–	2.6×10^4	7.1×10^6	–
NSA laundered 20th	2.1×10^4	<10	99.9	2.6×10^4	<10	99.9
NSB laundered 20th	2.1×10^4	7.5×10^2	99.9	3.0×10^4	<10	99.9

Table 3. The body weights of rats by NSS treatment for acute dermal toxicity evaluation

Period	Male		Female	
	Control	Treatment	Control	Treatment
1 day dosing	274.4 ± 11.10 g	276.8 ± 9.28 g	224.8 ± 3.64 g	225.6 ± 3.95 g
2 day dosing	273.2 ± 12.82 g	272.2 ± 8.73 g	214.9 ± 4.86 g	221.1 ± 7.07 g
4 day dosing	288.6 ± 15.62 g	289.2 ± 8.61 g	228.5 ± 3.17 g	229.1 ± 9.82 g
8 day dosing	312.7 ± 18.82 g	312.7 ± 14.23 g	241.1 ± 5.30 g	241.5 ± 9.57 g

mode of the antifungal effect of NSS can be suggested (Park *et al.* 2006; Park *et al.* 2007; Kim *et al.* 2008; Kim *et al.* 2011). It is proposed that NSS is first observed by the fungus, after which the outer structure (SiO_2 layer) of the absorbed NSS is changed through the interaction between the fungus and NSS. Nano-Ag is then gradually released, which is small enough to pass through the transmembrane of the fungus to effect the latter's energy metabolism. The nano-Ag then destroys the membrane electron transport chain of the fungus. This may be due to the inactivation of cell wall sulphhydryl (S-H) group; the interaction with the thiol groups of proteins and complexes, which are essential for respiratory chain and membrane permeability; or the disruption of the membrane-bound enzymes and lipids, which can cause cell lysis (Kim *et al.* 2011).

3. Antimicrobial stability

In order to determine whether a fabric coated with nanosized silica-silver retains the antimicrobial activity provided by the nanosized silica-silver after laundering twenty times, the above laundry cycle was repeated twenty times. Viable cells for the same bacteria were counted, and the percentage reduction of viable cells was calculated according to above method. The results are given in Table 1, below. As shown in Table 2 the nanosized silica-silver, prepared according to the method described in Example 1, exhibited a reduction of 99.9% in viable cells both before and after the coated fabric specimen was laundered twenty times. These results indicate that the nanosized silica-silver imparts excellent antimicrobial activity to its processed products for a long period of time, even after repeated laundering.

In addition to NSS, the coating composition used in the above method, may further include an aromatic, a surfactant and a stabilizer. Also, the composition may include, in addition to the aromatic, surfactant and stabilizer, a humectant, a dispersing agent, a stabilizer, an inactivating agent, an adhesion enhancer, a permeating agent, and an antifoaming agent (Kim *et al.* 2008).

Table 4. The clinical signs of rats by NSS treatment for acute dermal toxicity evaluation

Period	Male		Female	
	Control	Treatment	Control	Treatment
1 day Normal	5/5	5/5	5/5	5/5
2 day Normal	5/5	5/5	5/5	5/5
4 day Normal	5/5	5/5	5/5	5/5
8 day Normal	5/5	5/5	5/5	5/5

4. Acute dermal toxicity

We also investigated the toxicity of silica hybrid silver nanoparticles in rats. The skin of rats was treated with a 30 ppm nanoparticles solution (2 ml Kg^{-1}) for 24 hrs. No toxicity was detected in the treatment for 8 days (Tables 3 and 4). These results suggest that the fiber products coated with the silica hybrid silver nanoparticles can be used as strongly effective growth inhibitors in various microorganisms as friendly environmental textiles.

CONCLUSION

It has been suggested that fiber products coated with a composition comprising nanosized silica-silver particles, in which nano-silver is bound to silica molecules and a water-soluble polymer, retain excellent antimicrobial activity before and after laundering. Thus, nanosized silica-silver has a wide spectrum of applications in the fiber industry. The antimicrobial fiber product was provided based on an antimicrobial composition for coating fiber products comprising nanosized silica-silver particles in which nano-silver is bound to silica molecules and a water-soluble polymer, and the nanosized silica-silver particles are prepared by irradiating a solution comprising silver salt, silicate and the water-soluble polymer with radiation rays. The antimicrobial fiber product controlled bacteria but had no toxicity in the rat skin. The fiber product has high stability in washing and drying conditions (at least 20 washing and drying cycles).

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