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Short communication

Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles

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Abstract

Nanoparticle susceptibility constants were defined and used to evaluate the antimicrobial characteristics of silver and copper nanoparticles against *Escherichia coli* and *Bacillus subtilis*. Reaction of copper nanoparticles of 100 nm with *B. subtilis* showed the highest susceptibility ($Z = 0.0734$ mL/µg) whereas the reaction of silver nanoparticles of 40 nm with *E. coli* showed the lowest one ($Z = 0.0236$ mL/µg).
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Silver and copper are traditionally well-known antimicrobial materials. It is believed that these metals react with proteins by combining the -SH groups of enzymes; consequently, this reaction leads to the inactivation of the proteins (Jeon et al., 2003). When these metals are prepared in the form of very small particles, they are expected to show better antimicrobial characteristics because of their larger specific surface area. With the developments of nanotechnology, these metals were developed in the forms of nanoparticles and their antimicrobial characteristics were investigated in this study and in many studies. Sondi and Salopek-Sondi (2004) investigated the antimicrobial effect of silver nanoparticles against *Escherichia coli* and Hsiao et al. (2006) investigated the antimicrobial activities of copper-containing nanostructures. Through the results of TEM analysis and proteomic

research, it is believed that the silver nanoparticles interact with the elements of bacterial membrane, causing structural change, dissipation of the proton motive force, and finally cell death (Sondi and Salopek-Sondi, 2004; Lok et al., 2006). The nano antimicrobial metals can be used effectively by coating them on the surfaces that require antimicrobial functions, for instance, in medical devices and water treatment filters (Park and Jang, 2003; Morrison et al., 2006). However, there have been only few quantitative studies that could contribute to the comparative study of antimicrobial activities of metal nanoparticles.

In industrial applications of antimicrobial materials, numerical models and quantitative parameters are necessary for design optimization, performance evaluation and life-time prediction of antimicrobial systems. As one of the quantitative parameters, the susceptibility has been applied in numerical models to evaluate the antimicrobial effects of an upper-room UVGI (Ultraviolet germicidal irradiation) system against airborne microorganisms (Beggs et al., 2006). In this study, the

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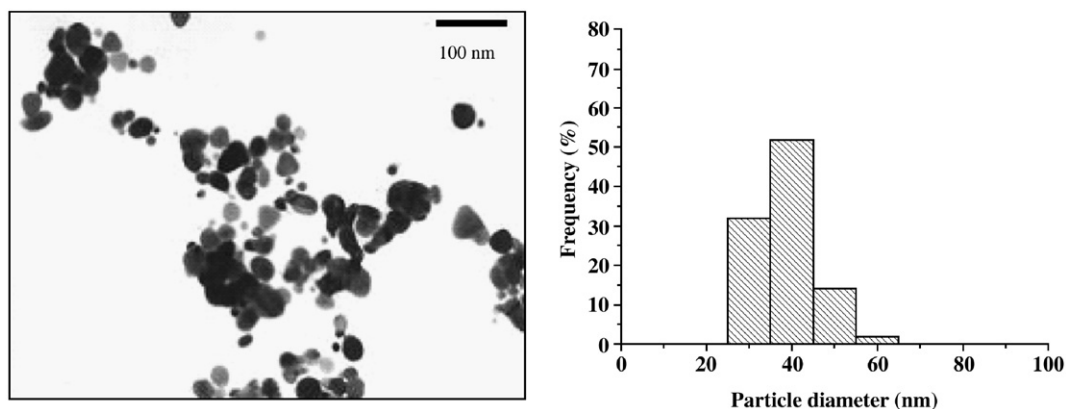*E-mail address:* hwangjh@yonsei.ac.kr (J. Hwang).

Fig. 1. TEM image of silver nanoparticles and their size distribution histogram.

nanoparticle susceptibility constant is suggested as a quantitative parameter for the estimation of antimicrobial activities of silver and copper nanoparticles. The nanoparticle susceptibility constant Z (mL/µg) is defined by the following equation:

$$Z = \frac{-\ln(N/N_0)}{C} \qquad (1)$$

where N is the bacterial colony forming units (CFUs) on the agar plate containing nanoparticles, N_0 is the CFUs on the pure agar plate, and C is the concentration of nanoparticles (µg/mL). Using the Z value and a given C value, the survival fraction (N/N_0) can be predicted. A higher Z value means that the bacteria are more sensitive to the nanoparticles, indicating that the nanoparticles are more effective in antimicrobial activity.

To examine the antimicrobial effects of silver and copper nanoparticles, approximately 200 CFUs of E. coli and B. subtilis (vegetative cells) were cultured on a Luria–Bertani (LB) agar plate and a Nutrient agar plate, respectively. In each agar plate, commercial silver (40 nm, ABC Nanotech Co. Ltd.) or copper (100 nm, Nano Technology Inc.) nanoparticles of concentrations of 10 to 100 µg/mL were supplemented. Figs. 1 and 2 show the microscopic images and histograms of particle size distributions of silver and copper nanoparticles. In each case, a nanoparticle-free agar plate cultured under the same condition as the nanoparticle-supplemented agar plate was used as the control. The numbers of colonies on the agar plates for E. coli and B. subtilis were counted after incubation for 24 h at 37 °C and 30 °C, respectively.

The experimental results are shown in Figs. 3 and 4. The survival fraction of bacteria (N/N_0) against nanoparticles decreased with increasing the concentration of nanoparticles. E. coli and B. subtilis were completely inhibited at the concentration higher than 70 µg/mL and 60 µg/mL for silver and copper nanoparticles, respectively. At each concentration of silver nanoparticles, the silver nanoparticle susceptibility constant (Z value) was determined by Eq. (1), and the Z values for a
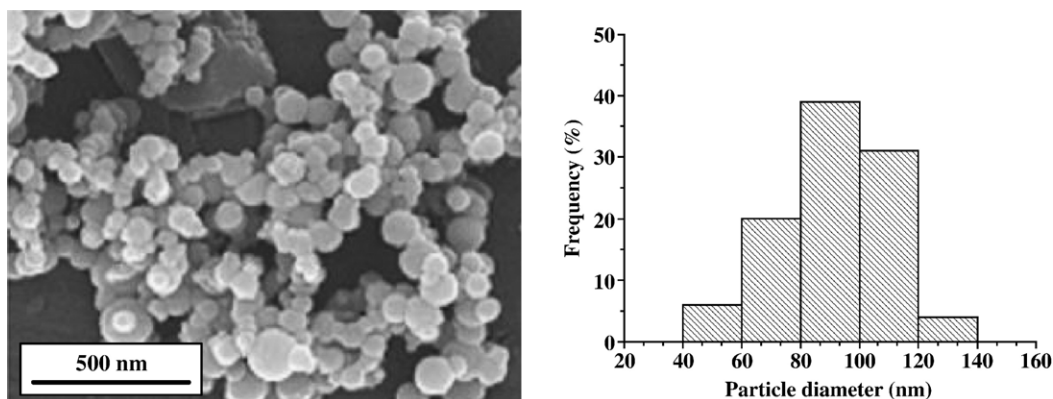

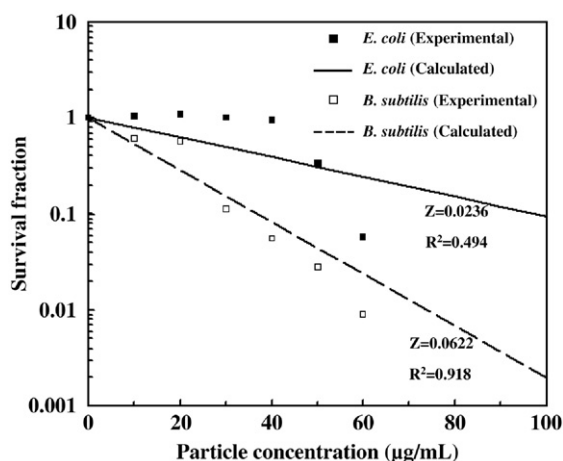Fig. 2. SEM image of copper nanoparticles and their size distribution histogram.

Fig. 3. Antimicrobial results of silver nanoparticles.

range of silver nanoparticle concentration were averaged. The averaged Z values of $E.$ $coli$ and $B.$ $subtilis$ to silver nanoparticles were 0.0236 and 0.0622 mL/µg, respectively. In the case of copper nanoparticles, the averaged Z values were 0.0574 and 0.0734 mL/µg for $E.$ $coli$ and $B.$ $subtilis$, respectively. The results in Figs. 3 and 4 show that $B.$ $subtilis$ was more sensitive than $E.$ $coli$ to the nanoparticles, meaning that $E.$ $coli$ was more resistive to nanoparticles than $B.$ $subtilis$ was. One possible explanation for the lower sensitivities of $E.$ $coli$ is that the outer membrane of Gram-negative bacteria such as $E.$ $coli$ is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective resistive barrier against nanoparticles (Brayner et al., 2006; Fan et al., 2002).

Curve fits in Figs. 3 and 4 were obtained by substituting the experimentally determined susceptibility constants into Eq. (1) and then calculating the
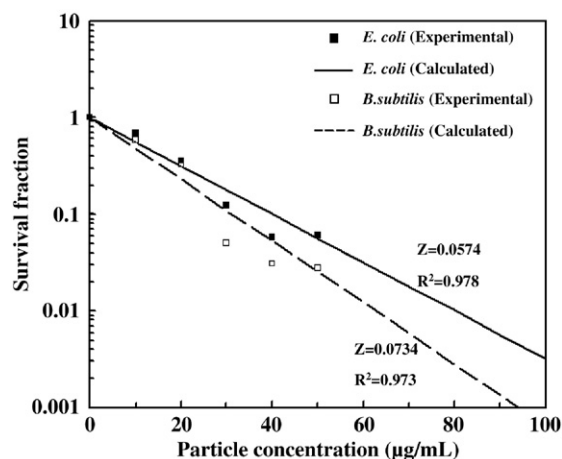

Fig. 4. Antimicrobial results of copper nanoparticles.

corresponding survival fractions. The correspondences of curve fits with experimental results are represented by R^2 values. The reaction of $E.$ $coli$ with copper showed the highest correspondence ($R^2 = 0.978$) while the reaction of $E.$ $coli$ with silver showed the lowest one ($R^2 = 0.494$). In the results of silver nanoparticles, there were threshold concentrations of 40 µg/mL and 20 µg/mL for $E.$ $coli$ and $B.$ $subtilis$, respectively. Exponential model using the susceptibility constant can not thoroughly reflect the information about the threshold concentrations. The silver nanoparticles which have threshold concentrations showed the lower correspondences than copper nanoparticles.

Table 1 shows the nanoparticle concentrations required for 90% antimicrobial efficiency, C_{90}, obtained for survival fraction $N/N_0 = 0.1$. Experimental C_{90} were determined by linear interpolation of experimental results. Differences between experimental and calculated C_{90} were less than 20% except for the case of $E.$ $coli$ reaction against silver nanoparticles.

In conclusion, nanoparticle susceptibility constants for silver and copper nanoparticles were clearly defined and used to evaluate the antimicrobial characteristics of these nanoparticles against $E.$ $coli$ and $B.$ $subtilis$. From the experimental results, susceptibility constants were determined and $B.$ $subtilis$ showed higher sensitivity than $E.$ $coli$ to both silver and copper nanoparticles. In addition, susceptibility constants were used for determining the nanoparticle concentration required to achieve a target antimicrobial efficiency. Susceptibility constant is beneficial in establishing evaluative references of antimicrobial nanoparticles and can be applied to develop analytical models for comparative studies of various antimicrobial systems. The nanoparticle susceptibility constants used in this study are limited to the sizes of 40 nm and 100 nm for silver and copper nanoparticles, respectively. Since the size of nanoparticles can effect on their antimicrobial activities, further study needs to be done about the relations between the size of nanoparticles and susceptibility constants.

Table 1
Concentration of nanoparticles required for 90% antimicrobial efficiency

Nanoparticles	Silver		Copper	
Bacteria	*Escherichia coli*	*Bacillus subtilis*	*Escherichia coli*	*Bacillus subtilis*
Experimental C_{90}(µg/mL)	58.41	32.12	33.49	28.20
Calculated C_{90} (µg/mL)	97.57	37.02	40.11	31.37
% error	67.04	15.26	19.77	11.24

References

Beggs CB, Noakes CJ, Sleigh PA, Fletcher LA, Kerr KG. Methodology for determining the susceptibility of airborne microorganisms to irradiation by an upper-room UVGI system. J Aerosol Sci 2006;37:885–902.

Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F. Toxicological impact studies based on Escherichia coli bacteria in ultra ZnO nanoparticles colloidal medium. Nano Lett 2006;6:866–70.

Fan L, Song J, Hildebrand PD, Forney CF. Interaction of ozone and negative air ions to control microorganisms. J Appl Microbiol 2002;93:144–8.

Hsiao MT, Chen SF, Shieh DB, Yeh CS. One-pot synthesis of hollow Au_3Cu_1 spherical-like and biomineral botallackite $Cu_2(OH)_3Cl$ flowerlike architectures exhibiting antimicrobial activity. J Phys Chem B 2006;110:205–10.

Jeon HJ, Yi SC, Oh SG. Preparation and antibacterial effects of Ag-SiO_2 thin films by sol-gel method. Biomaterials 2003;24:4921–8.

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, et al. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res 2006;5:916–24.

Morrison ML, Buchanan RA, Liaw PK, Berry CJ, Brigmon RL, Riester L, et al. Electrochemical and antimicrobial properties of diamondlike carbon-metal composite films. Diam Relat Mater 2006;15:138–46.

Park SJ, Jang YS. Preparation and characterization of activated carbon fibers supported with silver metal for antibacterial behavior. J Colloid Interface Sci 2003;261:238–43.

Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci 2004;275:177–82.