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The study of antimicrobial activity and preservative effects of nanosilver ingredient

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Abstract

In this study, we investigated the antimicrobial activity of silver nanoparticles (Ag-NPs) and platinum nanoparticles (Pt-NPs) aqueous solution, which were prepared using different stabilizer, such as sodium dodecylsulfate (SDS) and poly-(*N*-vinyl-2-pyrrolidone) (PVP), for *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E.coli*) by measuring the minimum inhibitory concentration (MIC). Antimicrobial effect of Ag-NPs for *S. aureus* and *E. coli* was investigated using cup diffusion method. The growth of Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were inhibited by Ag-NPs. The MIC of Ag-NPs for *S. aureus* and *E. coli* were 5 and 10 ppm, respectively. But the Au-NPs stabilized with SDS did not show antimicrobial activity. Also, the Pt-NPs stabilized with PVP (or SDS) did not show antimicrobial activity for the test organisms.

Keywords: Silver nanoparticles; Platinum nanoparticles; Stabilizer; Antimicrobial activity; Disinfection

1. Introduction

Human beings are often infected by microorganisms such as bacterium, mold, yeast, virus, etc., in the living environment. Research has been intensive in antibacterial material containing various natural and inorganic substances [1]. Among them, silver or silver ions have long been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities [2,3]. Several proposals have been developed to explain the inhibitor effects of silver ion/silver metal on bacteria. It is generally believed that heavy metals react with proteins by combining the thiol (–SH) groups, which leads to the inactivation of the proteins [4]. Recent, microbiological and chemical experiments implied that interaction of silver ion with thiol groups played an essential role in bacterial inactivation [5]. Also, it is revealed that bulk silver in an oxygen-charged aqueous media catalyzes the complete destructive oxidation of microorganisms [6]. However, the antimicrobial effects of silver nanoparticles (Ag-NPs) were not fully investigated. Metal nanoparticles (Me-NPs), which have a high specific surface area and a high fraction of surface atoms, have been studied extensively due to their unique physicochemical characteristics such as catalytic activity, optical properties, electronic properties, antimicrobial activity, and magnetic properties [7]. It can be expected that the high specific surface area and high fraction of surface atoms of Ag-NPs will lead to high antimicrobial activity compared to bulk Ag metal.

The purpose of this study was to examine the antibacterial activity of silver nanoparticles (Ag-NPs) and platinum nanoparticles (Pt-NPs) against *Staphylococcus aureus* and *Escherichia coli*.

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2. Experimental

2.1. Materials, bacterial strain, and culture condition

Colloidal Ag-NPs (0.054%, average diameter: 10 nm) solution and Pt-NPs (0.054%, average diameter: 3 nm) solution containing poly-(*N*-vinyl-2-pyrrolidone) (PVP) and sodium dodecylsulfate (SDS) as a stabilizer were prepared using ultrasonic irradiation method [8,9]. *S. aureus* (KCTC 1928) and *E. coli* (KCTC 1041) were used as a Gram-positive and Gram-negative bacterium, respectively. For the antimicrobial activity measurement, bacteria cultures were incubated at 35 °C in Luria medium (tryptone 1%, yeast extract 0.5%, sodium chloride 1%, agar 1.5%, Difco). The concentration of the bacteria was controlled from 10^7 to 10^8 CFU/mL.

2.2. Measurement of antibacterial activity

Ten microliters (5.4 ppm) of Ag-NPs (0.054%) or Pt-NPs (0.054%) was added in LB agar medium, which have 8 mm diameter hole in the center. The LB medium was incubated at 35 °C for 24 h. The antibacterial effects of Ag-NPs and Pt-NPs were measured by cup diffusion method (growth suppression exchange = total growth suppression exchange − diameter of the disc (8 mm)).

2.3. Measurement of minimum inhibitory concentration (MIC)

Different concentration of Ag-NPs (0, 5, 10, 50, 100 ppm) and Pt-NPs (0, 5, 10, 50, 100 ppm) was added in LB medium. Each bacterium culture (*S. aureus* and *E. coli*) was controlled at 10^5–10^6 CFU/mL and incubated at 35 °C. To establish the antimicrobial activity of Ag-NPs (or Pt-NPs) on the bacterial growth, the minimum inhibitory concentration of Ag-NPs and Pt-NPs for *S. aureus* and *E. coli* was determined by optical density of the bacterial culture solution containing different concentration of each nanoparticles after 24 h.

2.4. Measurement of colony-forming unit (CFU)

A bacterial culture medium containing Ag-NPs or Pt-NPs solution was diluted by adding sodium chloride (0.85%) solution to control the osmotic pressure of bacteria, and then spared on LB medium. Each bacterium (*S. aureu* and *E. coli*) was incubated its cultivation temperature for 48 h after that colony-forming unit (CFU) was measured. The Ag-NPs (0, 10 ppm) or Pt-NPs solution (0, 10 ppm) stabilized with (2.5 mM) PVP or (2.5 mM) SDS were added LB medium. Moreover, to compare the effect of Ag-NPs and Pt-NPs, the PVP (2.5 mM) and SDS (2.5 mM) solution without Ag-NPs or Pt-NPs was added to LB medium. The LB medium was incubated same method of above measurement of MIC. The decrease of bacterial at each LB medium was measured.

2.5. Scanning electron microscopy (SEM)

The morphological changes of *S. aureus* and *E. coli* by Ag-NPs were observed with a scanning electron microscope (SEM). Strains were prepared by cutting the agar, fixed for a minimum of 3 h in 2.5% (v/v) glutaraldehyde (100 mM phosphate buffer solution, pH 7.2), and then fixed in 1% (w/v) osmium tetra oxide for 1 h. The agar blocks were dehydrated through a graded series of ethanol (30, 50, 60, 70, 80, 90, 95, and 100%; each level was applied twice for 15 min each time) and ethanol:isoamyl acetate (3:1, 1:1, 1:3, and 100% isoamyl acetate twice for 30 min). The agar blocks on grid were dried with a critical-point drier using liquid CO_2 and coated with gold-coater for 5 min. The coated samples were observed under JSM-5600LV with accelerating voltage of 10 kV.

3. Results and discussion

Transmission electron microscopy (TEM) images of Ag-NPs and Pt-NPs were recorded with a Hitach H-8100 Electron Microscope, operating at 200 kV. Samples were prepared by placing a drop of the colloidal Ag-NPs and Pt-NPs solution onto a carbon-coated copper grid on an underlying tissue paper, leaving behind a thin colloidal film. Intact Ag-NPs having an average particles size of 10 nm with size distribution of 8–15 nm were observed (Fig. 1a). The average size of the Pt-NPs is about 3 ± 1 nm and has a narrow distribution over small range (Fig. 1b).

The antibacterial activity Ag-NPs and Pt-NPs solution stabilized with PVP for *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) was measured by cup diffusion method. The antibacterial activities of Pt-NPs stabilized with SDS or PVP were not observed. Also, the antibacterial activities of Ag-NPs stabilized with SDS were not observed. On the other hand, the Ag-NPs stabilized with PVP show higher antibacterial activity. The growth inhibition ring of *S. aureus* and *E. coli* treated by Ag-NPs stabilized with PVP was 9 and 4 mm, respectively (Fig. 2B). Ag-NPs solution stabilized with PVP shows good antibacterial activity for both *S. aureus* and *E. coli* compared with Ag-NPs stabilized with SDS (or Pt-NPs).

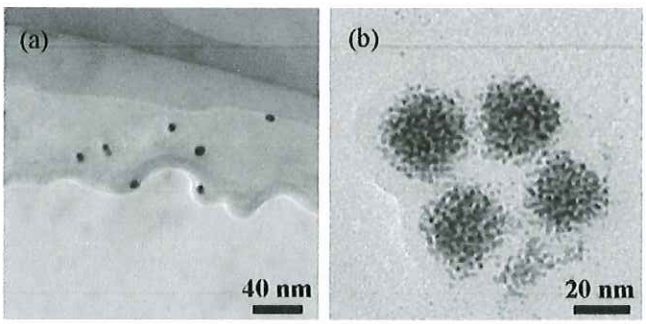

Fig. 1. TEM images obtained for (a) Au-NPs and (b) Pt-NPs stabilized with PVP.

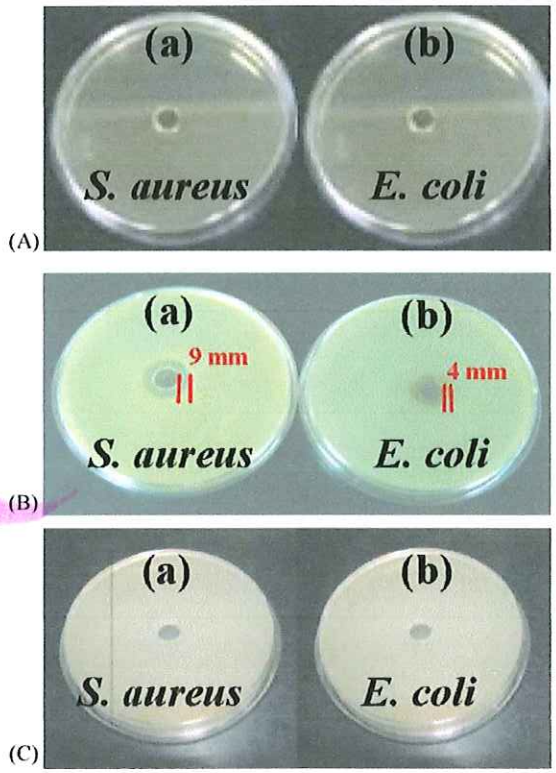

Fig. 2. Antibacterial activity of (A) Pt-NPs solution stabilized with PVP, (B) Ag-NPs solution stabilized with PVP and (C) Ag-NPs solution stabilized with SDS against *S. aureus* (KCTC 1928) (a) and *E. coli* (KCTC 1041) (b). All the concentration of Pt-NPs and Ag-NPs are 10 μL (5.4 ppm).

The reason can be considered two kinds of possibility. One is that the surface negative charge of SDS interferes with absorption of microbial, which has negative charge, on the surface of Ag-NPs. The other is that remaining or dissolved Ag ions in Ag-NPs solution interact directly with negative charge of SDS. This electrostatic effect prevents the interaction between Ag ion and microbial.

To investigate growth inhibition effect of Ag-NPs solution against *S. aureus* and *E. coli*, we measured the MIC and the changes of bacterial counts by different concentrations of Ag-NPs. The inhibition kinetics of bacteria containing different concentration of Ag-NPs solutions were investigated in *S. aureus* (Fig. 3) and *E. coli* (Fig. 4), respectively. The MIC of Ag-NPs for Gram-positive and Gram-negative was 5 ppm (*S. aureus*) and 10 ppm (*E. coli*), respectively. When *S. aureus* was exposed to different concentration of Ag-NPs solution, Ag-NPs solution of 10 and 20 ppm was enough to inhibit all viable cells of *S. aureus* within 3.3 and 4 h, respectively. When *E. coli* was exposed to different concentration of Ag-NPs solution, Ag-NPs solution of 10 and 20 ppm was enough to inhibit all viable cells of *E. coli* within 2.5 and 3.5 h, respectively. However, Ag-NPs solution of 5 ppm was not enough to inhibit both *S. aureus* and *E. coli* within 5 h. From the MIC results, the Ag-NPs stabilized with PVP showed higher inhibition kinetics against Gram-negative (*E. coli*) compared to Gram-positive (*S. aureus*).

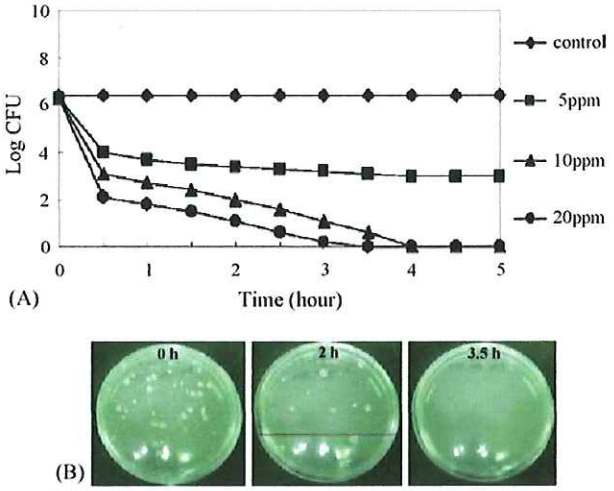

Fig. 3. (A) Growth inhibition curves of *S. aureus* in LB medium with different concentrations of Ag-NPs solution and (B) inhibition of colonies after Ag-NPs (10 ppm) treatment as the incubation time (0, 2, 3.5 h).

The Ag-NPs and Pt-NPs solutions stabilized with PVP show good growth inhibition effect for both *S. aureus* and *E. coli* as much as 99.99% (as shown in Table 1). It is worthwhile mentioning that all these Me-NPs (Ag-NPs and Pt-NPs) solution were quit stable for several months without observable aggregation. The Me-NPs stabilized with SDS are more stable than those of Me-NPs stabilized with PVP. Though, the stability of Ag-NPs stabilized with SDS is more stable than that of Ag-NPs stabilized with PVP, Ag-NPs stabilized with PVP show higher antimicrobial activity against Gram-negative and Gram-positive than that of Ag-NPs stabilized with SDS.

Fig. 5 shows the growth inhibition effect for different concentration of Ag-NPs stabilizer with PVP against *S. aureus* and *E. coli*. When *S. aureus* was exposed to different concentration of Ag-NPs solution, *S. aureus* was completely inhibited at 50 ppm of Ag-NPs concentrations. In the case

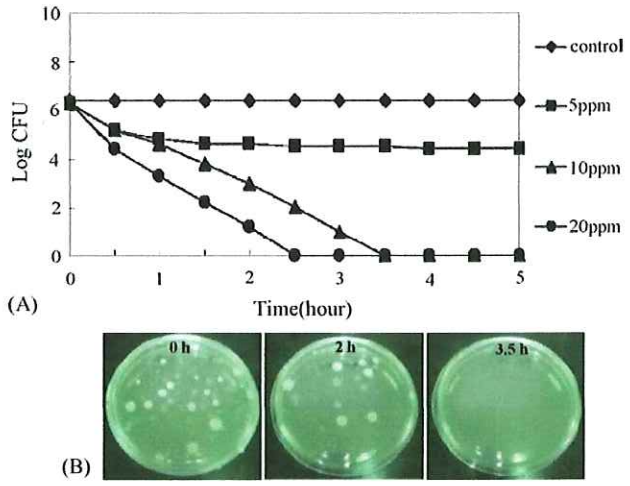

Fig. 4. (A) Growth inhibition curves of *E. coli* in LB medium with different concentrations of Ag-NPs solution and (B) inhibition of colonies after Ag-NPs (10 ppm) treatment as the incubation time (0, 2, 3.5 h).

Table 1
Growth inhibition rates of Ag-NPs against *S. aureus* and *E. coli*

Sample	Growth inhibition rate (%)	
	S. aureus (Gram-positive)	*E. coli* (Gram-negative)
Ag-NPs stabilized with PVP (10 ppm)	99.99	99.99
Ag-NPs stabilized with SDS (10 ppm)	0	0
Pt-NPs stabilized with PVP (10 ppm)	0	0
Pt-NPs stabilized with SDS (10 ppm)	0	0

Growth inhibition rate (%) = {(CFU/mL of control medium − CFU/mL of Ag-NPs or Pt-NPs solution treated medium)/(CFU/mL of control medium)}100.

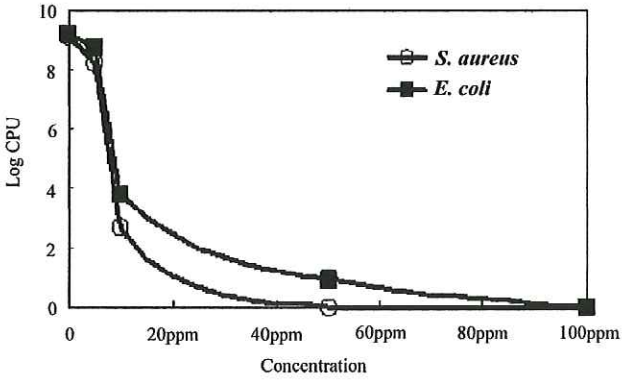

Fig. 5. Growth inhibition effect for different concentration of Ag-NPs stabilized with PVP against *S. aureus* and *E. coli*.

of *E. coli*, *E. coli* was completely inhibited at 100 ppm of Ag-NPs concentrations (Fig. 5). This result supports that one possible reason for the antibacterial activity of Ag-NPs might be their adsorption on bacterial surface. In general, Ag ions, which have antimicrobial activity, were used as an antibacterial agent. The antibacterial activity of Ag ion is inhibition of intracellular enzyme activity. Therefore, the other possibility can be considered that remaining Ag ions in Ag-NPs solution or dissolved Ag ions might affect on bacterial growth. However, the Ag-NPs stabilized with SDS have no antibacterial activity. The reason is that the surface negative charge of SDS interferes with absorption of microbial on the surface of Ag-NPs or Ag ion by electrostatic effect.

To investigate antimicrobial activity for *S. aureus* and *E. coli* by Ag-NPs, the morphology changes of *S. aureus* and *E. coli* by Ag-NPs solution were investigated with SEM. *S. aureus* (spherical shaped body) and *E. coli* (rod shaped bacteria)show their unique shapes (Fig. 6A). Although the strains of *S. aureus* and *E. coli* were still present (Fig. 6B), most strains of *S. aureus* and *E. coli* were damaged and extensively disappeared by addition of Ag-NPs solution. The surface of the cell walls of *S. aureus* was covered with substance resulted from the cell disruption after the Ag-NPs treatment.

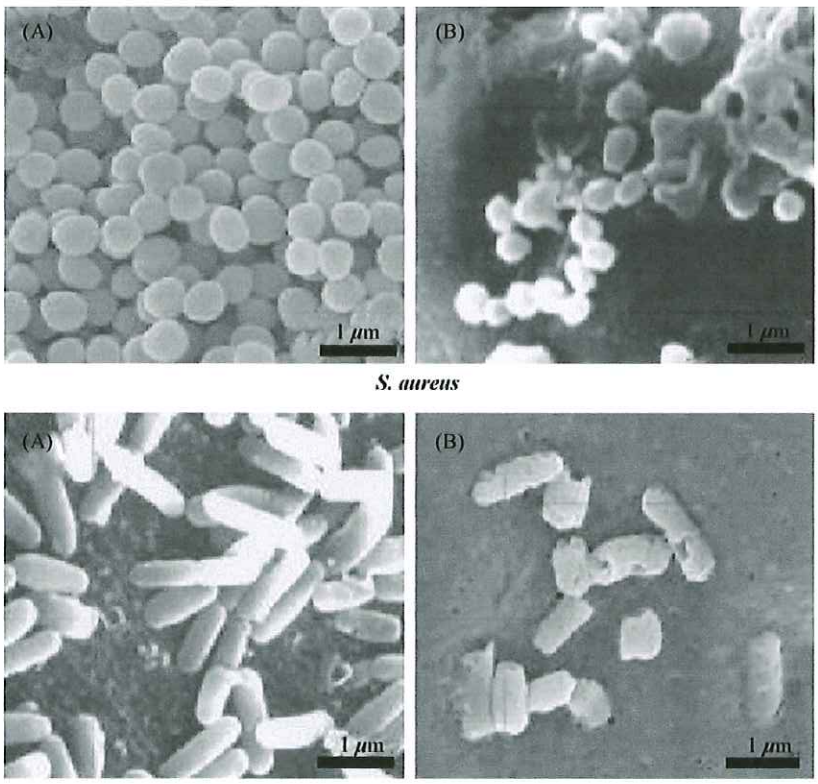

Fig. 6. Scanning electron microscopy of *S. aureus* and *E. coli*. Normal cells (A) and cells (B) grown on LB agar containing Ag-NPs solution (10 ppm).

The surface of the cell walls of *E. coli* treated with Ag-NPs was severely disrupted compared to the non-treated *E. coli*. It indicated that the Ag-NPs have an antimicrobial activity against *E. coli* and *S. aureus* by disrupting cells.

4. Conclusion

To investigate antimicrobial activity of metal nanoparticles (Ag, Pt) against *S. aureus* and *E. coli*, we prepared Ag-NPs and Pt-NPs solution stabilized with PVP or SDS. The Ag-NPs stabilized with PVP show strong antibacterial activity. The growth inhibition ring of *S. aureus* and *E. coli* treated by Ag-NPs stabilized with PVP were 9 and 4 mm, respectively. Minimum inhibitor concentration of Ag-NPs against the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) was 5 ppm and 10 ppm, respectively. *S. aureus* and *E. coli* were completely inhibited at 50 and 100 ppm of Ag-NPs concentrations, respectively. The surfaces cell walls of *S. aureus* and *E. coli* were disrupted by Ag-NPs.

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