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A comparative analysis of antibacterial activity, dynamics, and effects of silver ions and silver nanoparticles against four bacterial strains

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ABSTRACT

Although both silver ions and silver nanoparticles (AgNPs) have perfect antibacterial activity, but it was assumed that AgNPs have stronger activity than that of silver ions. In this study, we make a comparative analysis of activity, dynamics, and effects of silver ions and two types of AgNPs against four bacterial strains. The minimum inhibitory concentrations (MICs) of silver ions, AgNPs (I) and AgNPs (II) were 0.5, 1 and 2 μg/mL against *E. coli*, 1, 2 and 8 μg/mL against *P. aeruginosa*, 1, 2 and 4 μg/mL against *S. aureus*, and 1, 2 and 2 μg/mL against *S. epidermidis* respectively. This experimental results showed that Ag^{+} have stronger antibacterial activity than that of AgNPs (I) and AgNPs(II). Antibacterial dynamic curves revealed all the silver ions, AgNPs (I), and AgNPs (II) prolonged the growth lag phase of all four bacteria in a concentration-dependent manner. Furthermore, transmission electronic microscopy (TEM) observation showed that a major part of bacterial cells treated with 2 μg/mL of silver ion and AgNPs were destroyed within 5 h. The transmission electron microscopy (TEM) observation indicated that all the silver ions, AgNPs (I), and AgNPs (II) can induce severe damage in bacterial cells. The flagella of bacteria were damaged or even eliminated, which would cause movement disorders. Many holes or gaps were observed on cell surfaces, which would cause the leakage of cytoplasm and macromolecules, and leading to cell death at last. Our results suggested that silver ions have similar action mode and slightly better antibacterial activity than that of AgNPs against bacterial cells.

1. Introduction

Silver ions and associated compounds are known to act as broad-spectrum antimicrobials and corrosion protection materials. This antimicrobial activity has been recognized since ancient times (Tsezos et al., 1995; Zhao and Stevens, 1998; Feng et al., 2000; Jung et al., 2008; Koizhaiganova et al., 2015), and silver ions have been widely used in the corrosion protection (Silver et al., 2001; Squgo et al., 2015 Mirzaee et al., 2016), medical field for procedures such as dental work, catheterization, and the healing of burn wounds, to control pathogenic bacteria (Jung et al., 2008; Marambio-Jones and Hoek, 2010; Sclocchi et al., 2013; Abdollahi et al., 2015). In addition, a number of chemical forms of silver are known to be good antimicrobials, such as silver nanoparticles (AgNPs) (Kim et al., 2008, 2009; Sanghi and Verma, 2009; Sun et al., 2013; Shirakawa et al., 2013), silver sulfadiazine (Klasen, 2000; Silver, 2003), novel composite materials carrying silver (Kawashita et al., 2000; Kim and Kim, 2006; Yoon et al., 2008; Gutarowska et al., 2012a, 2012b; MacMullen et al., 2014). AgNPs, particularly, possess strong antimicrobial properties and have become an important area for research in the antimicrobial field. Many scientists believe that the antibacterial activity of AgNPs is greater than that of silver ions. For example, Lok et al. (2006) reported that the effective antibacterial concentrations of AgNPs and silver ions were in the nanomolar and micromolar ranges, respectively.

Surprisingly, our research found that silver ions have good antibacterial activity, sometimes even better than that of AgNPs. Although there have been several studies on the antibacterial effects of silver ions (Feng et al., 2000; Yamanaka et al., 2005; Jung et al., 2008; Rieger et al., 2016) or AgNPs (Sondi and Salopek-Sondi, 2004; Baker et al., 2005; Sharma et al., 2009; Jiraroj et al., 2014; Franci et al., 2015; Ahmed et al., 2016; Balakumaran et al.,

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2016; Shirakawa et al., 2016), their mechanism of action underlying these effects, their minimum inhibitory concentration (MIC) and antimicrobial dynamics have not been determined. To elucidate the antibacterial activity, dynamics, and effects of silver ions and AgNPs, and to compare the antimicrobial effects between silver ions and AgNPs, four bacteria strains, *E. coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, and *S. epidermidis* ATCC 12228, were selected as the research objects of bacteria in the environment for testing. The antibacterial properties of silver ions and two different types of AgNPs were evaluated based on the poisoned food technique, the antibacterial dynamic curves were determined, and cells were observed by transmission electron microscopy (TEM).

2. Materials and methods

2.1. Chemical reagents, microorganisms, mediums, and cultivation

Silver nitrate ($AgNO_3$) was purchased from Shanghai Institute of Fine Chemical Materials (Shanghai, China). $AgNO_3$ was suspended in deionized water and the concentration of silver ions was determined by ICP-Mass Spectrometry (Agilent 1260-7700e). Next, a standard solution of Ag^+ was prepared. The AgNPs solutions of AGS-WMB1000C (I) and AGS-WM 2000 (II) were purchased from Shanghai Huzheng Nanotechnology Company limited (Shanghai, China). The average diameter size of AgNPs (I) and (II) are 5 nm and 20 nm, respectively. The content densities of AgNPs (I) and (II) were 1000 and 2000 μg/mL, respectively. The size and morphology of AgNPs (I) observed by TEM was shown in our previous article (Li et al., 2010), and that of AgNPs (II) is shown in Fig. 1. Bacterial strains *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, and *S. epidermidis* ATCC 12228 were purchased from American Type Culture Collection (ATCC) and were maintained in our laboratory. Mueller-Hinton (MH) medium and Mueller-Hinton agar (MHA) medium, used for aerobic culture of the four bacterial strains at 37 °C, were the same as used in our previous work (Li et al., 2014). All solvents and reagents were of analytical grade.

2.2. Antibacterial activities of Ag^+, AgNPs (I) and AgNPs (II) against four bacterial strains

Antibacterial activity was measured by the poisoned food technique, described in our previous study with slight modifications (Li et al., 2013). The experimental Ag concentrations (μg/mL) of silver ions, AgNPs (I), and AgNPs (II) were all 0 (control), 0.125, 0.25, 0.5, 1, 2, 4, and 8 respectively. The quantity of *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* cells in each plate was approximately 10^6 colony-forming units (CFU). The plates were incubated at 37 °C in an incubator for 2 days. The MICs of silver ions, AgNPs (I) and AgNPs (II) against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* were determined after incubation for 2 days. Two separate experiments were performed in triplicate.

2.3. Antibacterial dynamics of Ag^+, AgNPs (I) and AgNPs (II) against four bacteria strains

The antibacterial dynamics of silver ions, AgNPs (I) and AgNPs (II) against *E. coli, P. aeruginosa, S. aureus,* and *S. epidermidis* were determined based on methods described in our previous study (Li et al., 2011) with slight modifications. The experimental Ag concentrations (μg/mL) of silver ions, AgNPs (I), and AgNPs (II) used in this study were all 0, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, and 8 respectively. Cultures (separately including *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*) were seeded into 96-well plates at a cell concentration of 10^6 CFU/mL. Next, each experimental group was incubated at 37 °C with shaking at 150 rpm in an automated growth curve analysis system (Bioscreen C). Antibacterial dynamic curves were drawn based on the absorbance at OD_{600} determined by the automated growth curve analysis system. The experiments were performed in triplicate.

2.4. Morphological alterations of four bacteria treated with Ag^+, AgNPs (I) and AgNPs (II)

The methods for observing alterations in the morphological structures of the four bacterial strains after exposure to silver ions, AgNPs (I), and AgNPs (II) were the same as described in our previous study (Li et al., 2011, 2013) with slight modifications. The experimental concentrations (μg/mL) of Ag were all 0 (control) and 2. The concentrations of the four bacteria were each 10^8 CFU/mL. Cultures were incubated at 37 °C with 150 rpm in a water bath shaker for 5 h. Then, the bacterial cells were sampled and prepared for TEM (Hitachi H-7650) observation. The experiments were carried out in triplicate.

3. Results and discussion

3.1. Antibacterial activities of Ag^+, AgNPs (I) and AgNPs (II)

The MICs of Ag^+, AgNPs (I), and AgNPs (II) determined by the poisoned food technique, are shown in Table 1. After incubation for 2 d, *E. coli* colonies filled the control plates, while only dozens of

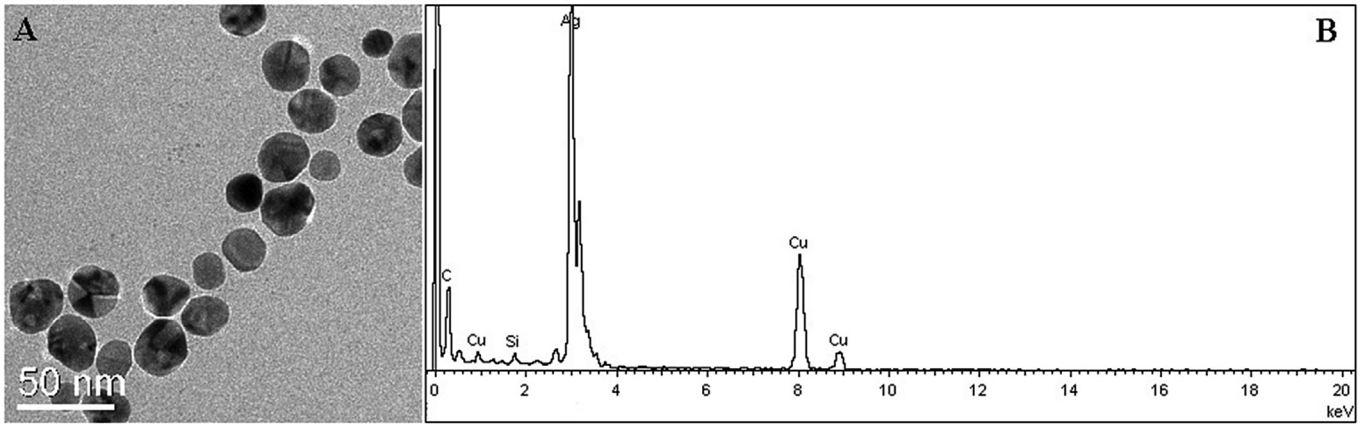

Fig. 1. Size and morphology observation of AgNPs (II) by TEM (A) and EDX analysis (B).