Antibacterial activity and intracellular accumulation of silver nanoparticles on *Escherichia coli* K-12

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ABSTRACT

As nanotechnology has achieved remarkable development, nanoparticles have been widely applied in many industries. Increase in nanoparticle usage, it is inevitable that engineered nanoparticles (ENPs) are exposed to environments such as water, soil, and air. Many researchers have emphasized the risk of ENPs. We exposed *Escherichia coli* K-12 to engineered silver nanoparticles (AgNPs) on to understand the antibacterial activities of AgNPs. We can compare the growth inhibition of AgNPs and Ag ions. The Ag ion was more toxic than AgNPs if they were compared as total Ag concentration. Transmission electron microscopy (TEM) was used to observe cell morphology and intracellular accumulation of AgNPs. AgNPs were found to interact with cell surface and significant amount of AgNPs were accumulated on *E.coli* K-12.

Keywords: Silver nanoparticles (AgNPs), Silver ion (Ag ion), Antibacterial activity, *Escherichia coli*, Intracellular accumulation.

1 INTRODUCTION

Nanoparticles have always existed in our environment, not only nature but also anthropogenic sources [1]. Development of nanotechnology there has been a rapid increase in interest the use of nanoparticles in worldwide. Engineered nanoparticles (ENPs) are desigened to achieve particular physic-chemical properties that relate to the product application. Specially silver nanoparticles (AgNPs) were shown to be an effective bactericide [2] due to their extremely large surface area, which provides better connect with microorganisms [3]. AgNPs are being increasingly used in broad commercial such as cosmetics [4], clothing [5], in medical area [6]. Besides, Silver ions (Ag ions) are already known to interact with thiol groups in proteins; this inhibits respiratory enzymes [7]. Silver ions exert an adverse effect on DNA replication and the structure of the cell membrane [8]. However, the mechanism of antibacterial action of AgNPs and Ag ions have not been explained clearly. Various combination of silver ion release followed by cellular uptake and direct interaction between AgNPs and cell membrane [9]. AgNPs might also act as a Troian horse, entering a cell by bypassing its barriers to nomal sized silver, and then releasing silver ions that damage to cell machinery [10]. The antibacterial activity of AgNPs was dominated by silver ions less than about 10 nm fine nanoparticles were employed that release high concentration of silver ions [11]. Therefore, researchers are ongoing debate for toxicity main effect are silver nanoparticles and how investigation of their antibiacterial mechanisms different on the microorganism. *Escherichia coli* have long been known as a bioreporter in ecotoxicology [12]. We investigated antibacterial activity of engineered AgNPs compare to Ag ions using *Escherichia coli* K-12. Moreover, the results of this study provide us useful understanding of antibacterial properties on the bacteria.

2 MATERIALS AND METHODS

2.1 Charicterization and preparation of AgNPs

The AgNPs used in this study was manufactured by Nanoleader, Ins, Korea. The each final total silver concentration was diluted in distilled water. Also, AgNO3 were purchased in Sigma-Aldrich (purity >99 %) and it diluted in DI water, each same total silver concentration were used as Ag ion. The Core size of AgNPs and their morphology were investegated by a TEM (JEOL 2100) at 200 kV. The hydrodynamic size was determined using dynamic light scattering (DLS) (Zetasizer Nano, Malvern). The concentrations of AgNPs were determined by ICP-MS analysis.

2.2 Bacterial strain and cultivation

In this study, the *Escherichia coli* K-12 (KCTC 1116) strain were used as a representative of gram-negative

bacteria. The bacteria were grown in Luria-Bertani liquid medium (Yeast extract 5 %, Trypton 5 %, and NaCl 10 %) at 37 ℃, 150 rpm for 12 h.

2.3 The bacterial growth inhibition test

To examine the growth inhibition of AgNPs and Ag ions, the cells were exposed to 0 to 30 mg/L of AgNPs and Ag ions. Optical density (OD) 600nm measurement by UV-Vis was used to determine the growth curves of AgNPs and Ag ions on the cells.

2.4 TEM sample preperation

Transmission electron microscopy (TEM) analysis used to observe the interaction between the AgNPs with the cell and intracellular shape. The *E. coli* were treated with 10 mg/L of AgNPs and incubated at 37 ℃ with shaking at 150 rpm for 12 h. For negative staining, the cell washed with a phosphate buffered saline (PBS) buffer solution. A 10 μl sample drop on TEM copper grids. To determine the intracellular image of TEM, the cells was collected by centrifugation at 1500 rpm, 5 min. The pellets were fixed in a mixture of 4% paraformaldehyde buffered with PBS buffer solution (pH 7.4). Postfixation was performed with 1% osmium tetroxide in the same buffer and washed three times in phosphate buffer following fixation. The dehydration in rising concentrations of ethanol and embedded in Poly/Bed 812-Araldite medium (Polysciences Inc., USA) using propylene oxide. The samples of thin section were double stained with uranyl acetate and lead citrate. After these treatments, the sections (less than 100nm) on the TEM coper grids and examined with a TEM (Tecnai G2) at 120 kV.

3 RESULTS

3.1 AgNPs characterization

Table 1. The characterization of AgNPs

Materials	pH	Core size by TEM	Hydrodynamic size by DLS
AgNPs	8.8	2.71 ± 1.25 nm	68.3 ± 1.76 nm

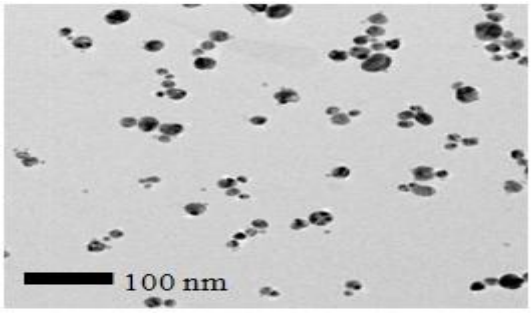

Figure 1: The TEM image of AgNPs.

For characterization of AgNPs, the image TEM analysis was done. The Fig.1 shows that silver to be nanosized and well dispersed in DI water. The core sizes of AgNPs are 2.71 ± 1.25 nm mesured by TEM. Furthermore, the hydrodynamic size determided were 68.3 ± 1.76 nm of AgNPs. Also the absorption spectrums of AgNPs are 412 nm and pH is 8.8 in the colloid. The concentration of AgNPs are 361± 8.25 mg/ in colloid solution.

3.2 The growth inhibition test

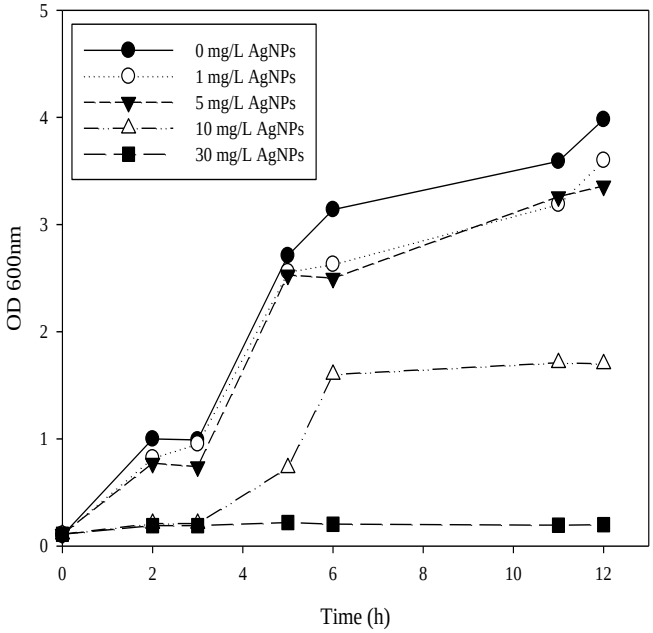

Figure 2: The growth curves of AgNPs total silver concentration (0 to 30 mg/L) on *Escherichia coli* K-12.

We investigated antibacterial ability of AgNPs and Ag ions using *E. coli* K-12. Non exposure (0 mg/L) of AgNPs and Ag ios used as a contorol experiment. In growth inhibition test, the *E. coli* was inhibited on the increasing of AgNPs concentration (Fig. 2). After exposure to 10 mg/L of AgNPs, the log phase were delay and did not grown well compare to non exposure to AgNPs on the cell. In addition, the *E. coli* K-12 were exposed to 30 mg/L of AgNPs could inhibit growth obviously compare to control of cells. However, the Ag ions were extremly ihibit the bacterial growth (Fig.3).

In this results, we can conclude that silver ion is more toxic than silver nanoparticles at the same concentration on *E. coli* K-12. Therefore, Ag ions were able to more effectively inhibit the growth of bacteria than AgNPs.

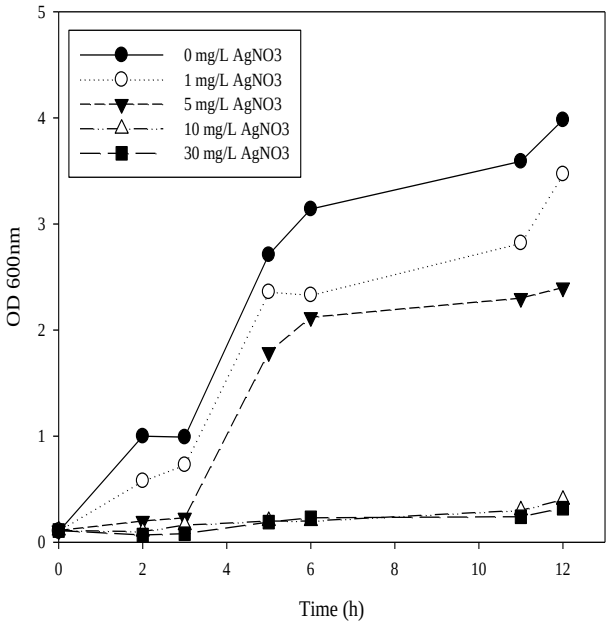

Figure 3: The growth curves of AgNO3 concentration (0 to 30 mg/L) as a silver ion on *Escherichia coli* K-12.

3.3 The TEM analysis of bacteria.

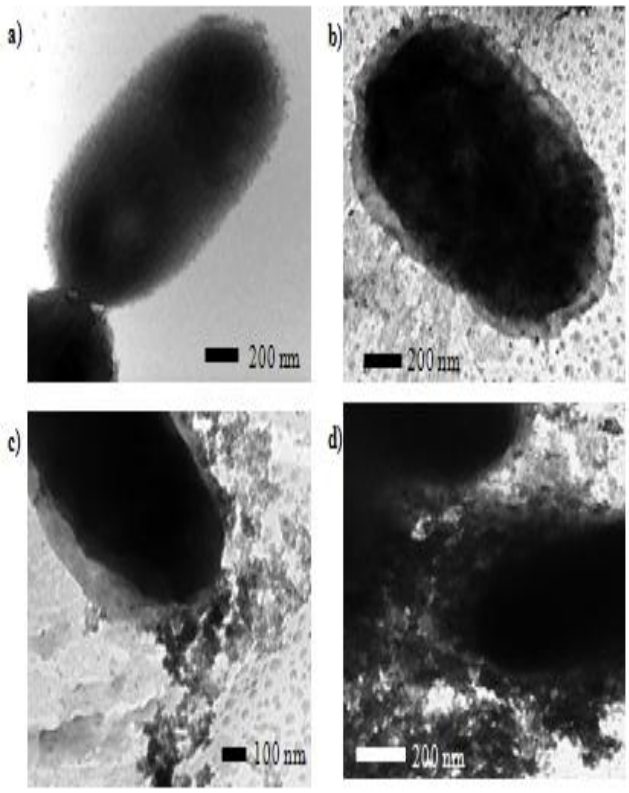

Figure 4: The TEM image of native cell (a) of *E.coli* K-12 and interaction with AgNPs 1 mg/L (b), 5 mg/L (c), 10 mg/L (d) in LB medium for 12h.

We can observe the TEM images of native cell shape and treated after AgNPs to the *E.coli* K-12 with a negative staining method (Fig. 4). The AgNPs can attach the cell surface and interaction with the bacteria. Exposed to 5 mg/L of AgNPs on the cells, the AgNPs were surrouned with cell membrane (Fig. 4. c). Obviously, significantly amount of AgNPs are surrounded with the bactera when exposed to 10 mg/L of AgNPs (Fig. 4. d). It would like to ready for effect to the intracellular organisms and willing to effect to the bacteria.

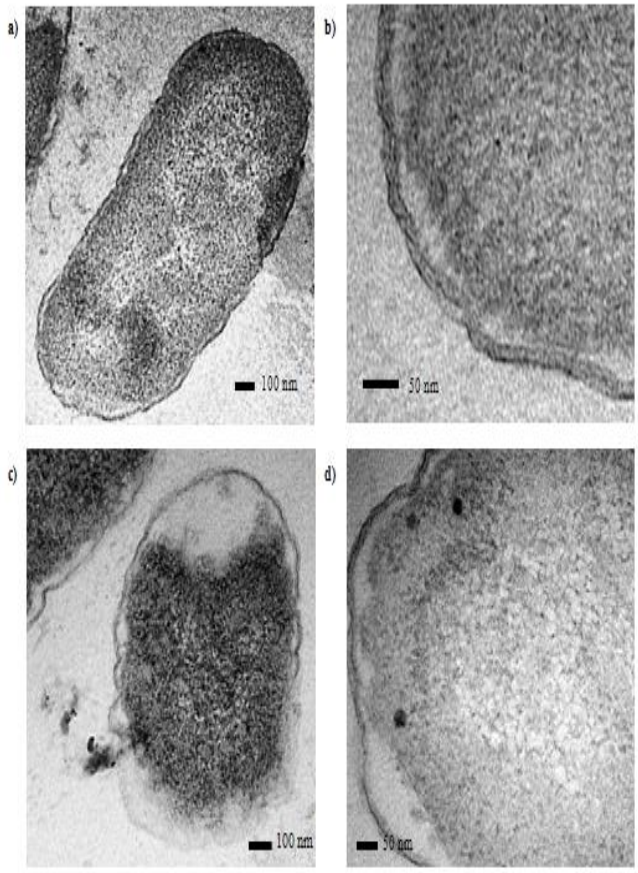

Figure 5: The sections of TEM image of native cell (a), their membrane (b) of *E.coli* K-12 and exposure to AgNPs 1 mg/L (c), and 5 mg/L (d) in LB medium for 12h.

In native cell as control, we observed the gram negative cell (Fig. 5. a) and inner organism constitution of cell such as outer membrane, peptidoglycan later and ribosomes (Fig. 5. b). Exposure to 1 mg/L AgNPs to *E. coli* K-12 for 12h, the AgNPs can interact with bacteria membrane (Fig. 5. c). In 5 mg/L AgNPs to *E. coli* K-12 it is doubtable whether AgNPs can penetrate the cell membrane and AgNPs aggregated in the inner cell (Fig. 5. d). Therefore, the energy dispersive spectroscopy (EDS) analysis will need for distinguish clearly between AgNPs and ribosomes. Besides, the AgNPs can go through the cell membrane and we will confirmed that cellular uptake of AgNPs in the cell.

4 CONCLUSION

Based on the results, AgNPs can significantly increase antibacterial activity with the increasing of concentration of AgNPs. After being exposed to 10 mg/L silver nanoparticles, the lag phase postponement was more evident and growth inhibition on *E.coli*. The high concentration of AgNPs is a important key parameters in evaluating the applicability. It will determine the effect of the agent to a great extent [13]. Also, AgNPs have a excellent antibacterial property on the bacteria and Ag ions. TEM analysis was used to observe the cell and intracellular accumulation of AgNPs. Therefore, AgNPs were found to significantly interaction with the cell surface. To determine cellular uptake of AgNPs, the AgNPs can penetrate into the cell. Finally, TEM-EDS analysis will be need for next research. In addition, the importance of released silver ion from silver nanoparticles effect to antibacterial ability and to evaluate the toxicity of nanoparticles. Additional works need to characterize the mechanism of silver nanoparticles and silver ions on the subcellular level. The nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, such as permeability and respiration [14]. The present results indicate that the particles could efficiently enter the cells by a Trojan-horse type mechanism. It seems to conclude that the AgNPs may also affect the potential intracellular toxicity on the bacteria. Toxicological investigations of AgNPs will deal with for the future studies include that NOM (Natural organic matter) and pH influence their antibacterial ability and their metabolism the toxicity of nanoparticles.

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