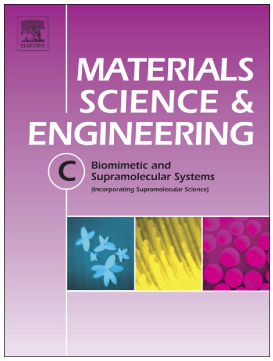

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The application of silver nano-particles on developing potential treatment for chronic rhinosinusitis: antibacterial action and cytotoxicity effect on human nasal epithelial cell model

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Abstract

Chronic rhinosinusitis (CRS) has raised attentions both in many countries due to its high relapsing rate and the resistance of certain treatments especially antibiotics application on both acute and chronic bacterial rhinosinusitis. The aim of this research is stressing on developing an effective alternative treatment for treating CRS and reducing the use of antibiotics to avoid further resistance forming. The antibacterial functions of silver nano-particles (AgNPs) are well known according to previous reports and studies. However, for developing a suitable treatment for further clinical application, a variety of AgNPs cell cytotoxicity experiments and AgNPs antibacterial properties experiments were examined *in vitro* in this study. For imitating the clinical condition of CRS, the human nasal epithelial cell line (RPMI2650) has chosen as experimental model. Moreover, Gram-positive *Staphylococcus aureus (S. aureus)* and Gram-negative *Escherichia coli (E. coli)* were selected for antibacterial function experiments. The analytical results demonstrated that 5 ppm of AgNPs not only maintains more than 80% of cell activity to RPMI2650, but also possesses more than 80% of antibacterial function to *S. aureus* and 100% of antibacterial function to *E. coli*. Therefore, 5 ppm of AgNPs might be considered as a promising antibacterial agent for treating CRS.

Keywords: Chronic rhinosinusitis; Silver nano-particles; Human nasal epithelial cell; Cytotoxicity; Antibacterial agent

1. Introduction

CRS has been considering as one of severe prevalent chronic health problems for both Western and Asian countries, which impacts all age groups of people due to its high relapsing rate. CRS is a condition which can be classified according to various symptoms of sinus inflammation with the time period at least two months or longer [1]. There are numerous factors can potentially induce rhinosinusitis including infection, allergy, inflammation or obstruction of the sinuses [1]. Moreover, in terms of bacteria-infected rhinosinusitis which covers four main kinds of bacterial strains such as *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Staphylococcus aureus* [2, 3]. Based on a meta-analysis study that collected data from 1990 to 2006, which involved various antibiotic treatment studies of acute bacterial rhinosinusitis in the United States, the result conducted a potential fact that *S. aureus* could be considered as the main pathogen in acute bacterial rhinosinusitis [2]. Additionally, a clinical outcome of research conducted by Niederfuhr et al. [4] indicated that *S. aureus* might play an important role with respect to CRS.

In terms of the pathogenesis of CRS which might due to unsolved bacterial-infected and inflammation on/inside nasal mucous membrane inside the nose [4]. A number of medical studies have applied the RPMI2650 cell line as testing model because of its high similar descriptions of human nasal mucous membrane cell including the function of yielding mucoid material on the cell surface, creating an enzymatic metabolic encumbrance, and also

possessing comparable permeability with human nasal mucosa [5].

Nowadays, antibiotics have been considered as one of common treatments at the first stage for treating acute bacterial rhinosinusitis. It is evident that the most common treatment for bacterial infection rhinosinusitis is prescribed antibiotics [6-8]. However, resistance of antibiotics is recognized as an issue that arises when an antibiotic has lost the ability to control or eliminate bacterial growth within human bodies from time to time [2, 6]. Additionally, untreated acute bacterial rhinosinusitis might possess high probability of developing into CRS due to uncompleted antibiotics therapy for treating sinusitis [6].

Ag is a well known element with potential germicidal functions which has applied as antibacterial agents by releasing as metal Ag^{+} ions or nano-particles [9-12]. AgNPs are considered as possessing the ability to perforate the bacterial cell wall and afterward run through it. Accordingly, inciting structural transforms in the cell membrane such as its permeability and death of the cell [11, 13]. A dedicate study conducted by Panáček et al. [6] which combined various antibiotics with low concentration AgNPs for improving antibacterial efficiency. The outcomes demonstrated that AgNPs increased the efficiency of antibiotics and decreased the usage amount of antibiotics for eliminating bacteria. Nevertheless, there was a lack of research that has stressed on investigating or applying the antibacterial capacities of AgNPs as a potential clinical antibacterial agent for especially treating CRS. Therefore, this research aims to develop a promising antibacterial agent of

treating CRS by applying different concentrations of AgNPs as the intermedium to inhibit *S. aureus* and using RPMI2650 cell groups as the examinational model. The antibacterial functions and cell cytotoxicity response of AgNPs will be examined in this study.

2. Materials and Methods

2.1 Preparation of AgNPs

The 100 ml of aqueous buffer solution with concentration of 20 ppm and diameter of 10 nm AgNPs was purchased from Sigma-Aldrich, Taiwan. The phase identification of AgNPs was analyzed by a high resolution transmission electron microscope (TEM, JEM-2100) equipped with an energy-dispersive X-ray spectrometer (EDS, INCA). The 20 μl suspension was pipetted onto copper grid coated with carbon film. Subsequently, the grid was dried thoroughly in an electronic dry cabinet and then observed at 200 kV. For cell cytotoxicity and antibacterial testing, the solution was diluted into different concentrations of 1, 3, 5 and 7 ppm and which were defined as group of AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7, respectively.

2.2 TEM observation of RPMI2650 cell cultured with AgNPs

To investigate the phenomenon of AgNPs entering RPMI2650 cell (ATCC CCL-30, Taiwan), the RPMI2650 cells were cultured first within formulated Minimum Essential Medium (MEM; Gibco, USA), and further, initial medium were supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS, Gibco USA) mixed with 1% Penicillin Streptomycin (P/S, Gibco USA) in a highly humidified atmosphere. Subsequently, the RPMI2650 cell suspensions with a 1×10^5 cells $m1^{-1}$ were cultured with 5 ppm of AgNPs (ultra-sonicated for 30 min) and without AgNPs in an incubator at 37°C with 5% CO_2 for 24 h,

respectively. Sample preparations for TEM were followed by fixing, dehydrating, resin embedding, ultra-microtome sectioning. Cross-sectional appearance of the samples was observed using the Hitachi HT-7700 TEM at 80 kV.

2.3 Morphology observation of RPMI2650 cell cultured with *S. aureus*

To evaluate the cell morphology of RPMI2650 when suffering the invasion of *S. aureus* (ATCC6538P, Taiwan), the RPMI2650 cells were cultured first within formulated medium as mentioned before. Meanwhile, *S. aureus* strain was grew and maintained in a formulated Tryptic Soy Broth (TSB, Acumedia USA) with anaerobic atmosphere in a dry anaerobic indicator strip (GasPak™, Becton, Dickinson and Company USA) under 37°C for incubating 24 h. For tracking the potential invasive methods of *S. aureus* on RPMI2650 cells, *S. aureus* had processed by fluorescein isothiocyanate before culturing with RPMI2650 cells. Hereafter, the 1×10^4 cells ml^{-1} RPMI2650 cell suspensions were cultured with 1×10^4 CFU ml^{-1} *S. aureus* and without *S. aureus* in an incubator at 37°C with 5% CO_2 for 1 h, respectively. Morphology observation was conducted by Olympus IX71 optical microscope (OM) and fluorescence microscope (FM).

2.4 Cell cytotoxicity assay

The cell cytotoxicity assay was followed by ISO 10993-5 specifications. The different concentrations of AgNPs (i.e. AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7) were ultra-sonicated for 30 min to avoid self-aggregation and were sterilized by sterile filtration

using syringe filters (pore size ≦ 0.22 μm). Next, added into culture medium then were gentle shaking at 37 °C for 24 h on an orbital shaker. Meanwhile, to culture the RPMI2650 cells with 1×10^5 cells $m1^{-1}$ to each well in a 96-well micro-plate. Subsequently, cultured with AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7 samples for three independent experiments (N = 3) at 37°C with 5% CO_2 for 24 h, respectively. Cells were then incubated in 50 μl of 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) solution for an additional 3 h to form the formazan solvent precipitates. Then, the MTT solution was decanted and 100 μl of isopropanol were added in each well. To sway the plate for 10 min to release the formazan and subsequently applied ELISA Reader (BioTek-Epoch, USA) with a 570 nm filter to read the optical density (OD) of cells. Zinc dibutyldithiocarbamate (ZDBC) as positive control and high density polyethylene (HDPE) as negative control, respectively. Control (blank) cells were exposed to FBS medium only.

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlates to the amount of blue-violet formazan formed, as monitored by the OD at 570 nm. The reduction of viability was calculated by Equation (1) which represented the cell activity:

$$\text{Viability } \% = OD_{570e} / OD_{570b} \quad (1)$$

Where,

OD_{570e} is the mean value of the measured OD of the test sample; OD_{570b} is the mean value of the measured OD of the blank. The sample has considered as acute cytotoxic potential when viability value of the sample is reduced to < 70 % of the blank.

2.5 Antibacterial evaluation

Before the examination, all experimental tools were sterilized at 120°C for 15 min by autoclaving. The bacterial strains of *S. aureus* which cultured in Tryptic Soy Broth (TSB, Acumedia USA), and *E. coli* (ATCC8739, Taiwan) which cultured in lysogeny broth (LB, Acumedia USA) were conducted in the tests. For *S. aureus*, nutrition agar plates were streaked out with a loop from the frozen stock and grown in anaerobic environment with GasPack system overnight at 37 °C, more so, *E. coli* were cultured under aerobic environment overnight at 37 °C. A single colony was incubated into nutrition broth in a conical flask and prepared the bacterial suspension at 1×10^5 CFU ml^{-1}. Subsequently, both bacterial *S. aureus* and *E. coli* suspensions were incubated with ultra-sonicated AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7 samples (N = 3) at 37 °C for 24 h under anaerobic and aerobic environment, respectively. After incubation, the colony of each petri-dish was counted to calculate the number of bacteria. The antibacterial rate (AR) determined by Equation (2) was considered as the antibacterial effect [11].

$$AR\ (\%) = 100 \times [N_{(control)} - N_{(sample)}] / N_{(control)} \quad (2)$$

Where, $N_{(control)}$ is number of bacteria adhering on control petri-dish after 24 h incubation, and

$N_{(sample)}$ is number of bacteria adhering on AgNPs medium contented petri-dish after 24 h incubation.

2.6 Statistical analysis

Data were expressed as mean ± standard error of the mean. Data were analyzed by analysis of variance using the Student's *t*-test. *P* values < 0.05 were considered statistically significant.

3. Results and discussion

3.1 Characterization of AgNPs

Fig.1 (a) shows a bright-field electron micrograph and the [011] zone axis selected-area electron diffraction pattern (SAEDP) of the original solution with AgNPs sample. Clearly, black spherical particles with ~10 nm were found in the sample. No other impurities or intermetallic compounds were observed in the sample. In addition, the ring spots in the SAEDP indicated the presence of nano-polycrystalline structure with mainly {111} crystalline plane in the sample. According to the *d*-spacing and camera length between the ring spots, and results of energy-dispersive X-ray spectroscopy (EDS) detection (Fig.1 (b)), it can be confirmed that the black spherical particles were the AgNPs with face centered cubic structure. This characteristic is similar to that observed in previous study [11, 14]. However, several studies have indicated that the high reactivity of high density Ag {111} facets [15-17]. Moreover, the reactivity of Ag for microorganisms has also been demonstrated that the reactivity is favored as the AgNPs accompany with high atom density facets such as {111}. The AgNPs found in the surface of the bacteria corroborates the faceting of the particles as well as the direct interaction of the {111} facets [14]. This feature could be attributed that AgNPs of small sizes (<10 nm) present electronic effects, which are reported to enhance the reactivity of the nanoparticle surfaces and a higher percentage of the surface area promote the binding strength of the particles to the bacteria [14, 18]. Therefore, a high reactivity of the

AgNPs played an important role in this study.

3.2 Cross-sectional TEM observation of the RPMI2650 cells cultured with AgNPs

Fig.2 (a) presents the TEM image of the control sample with RPMI2650 cells only. Apparently, the untreated RPMI2650 cells exhibited a typical structure with completely plasma membrane, nucleolus, organelles and cytoplasm. No black spherical-like metallic particles were observed on the external plasma membrane and inside the cells. However, Fig.2 (b) is the experimental sample with RPMI2650 cells and AgNPs together. In this image, the presence of aggregated black spherical-like particle clusters with a size around 100 nm (as indicated by arrows) can be found within the cells. Based on the size measurement analysis in the higher magnification image, the phenomenon of AgNPs entering RPMI2650 cells was confirmed. Similar TEM features could also be discovered in that of cellular gastrointestinal uptake behavior of gold nano-particles in mouse primary osteoblasts, phagocytic RAW 264.7 cells and nonphagocytic HepG2 cells [19, 20]. Thus, the images (Fig. 2(a) and 2(b)) of TEM demonstrated that AgNPs might possess the ability of passing through RPMI2650 cell membrane. Generally, nanoparticles with smaller size are more efficiently to enter and exit in the cell. Moreover, spherical-like nano-particles are more beneficial to be penetrated into the cell than other shape nano-particles [21].

3.3 FM observation of RPMI2650 cell cultured with *S. aureus*

Fig. 3(a) displays the image of RPMI2650 cell without adding *S. aureus* under OM and

Fig. 3(b) is the image of RPMI2650 cell without adding *S. aureus* under FM. The two images showed no bacteria observed. Nevertheless, Fig. 3(c) illustrates the image of the RPMI2650 cell after adding *S. aureus* for 1 h under OM. Numerous *S. aureus* were observed to adhere on the external cell membrane as indicated by arrows. Further investigate by FM, Fig. 3(d) presents the image of RPMI2650 cell after adding *S. aureus* for 1 h under FM. Clearly, the phenomenon of *S. aureus* entering the RPMI2650 cell can be observed as indicated by arrows. Therefore, the results demonstrated that *S. aureus* might possess capability of entering through the cell membrane of RPMI2650 after adding *S. aureus* for 1 h. It is well known that *S. aureus* could be considered as the main pathogen in acute bacterial rhinosinusitis [2]. This result proved that the *S. aureus* could significantly effect on the human nasal mucous membrane cell in a short-term culturing.

3.4 Investigation of cell viability

To assess the effect of increasing equivalent concentrations of AgNPs on RPMI2650 cells, the change of RPMI2650 cell morphology (Fig.4(a)) was observed by OM after adding different concentrations of AgNPs medium (AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7) into cells for 24 h. It is evident that RPMI2650 cells which might not present significant difference within different AgNPs concentrations when initially observed by OM. Furthermore, the MTT assay was performed for examining the OD reading under λ = 570 nm (Fig. 4(b)) and cell activity (Fig. 4(c)) with 24 h incubation. Both statistics indicated that all groups of

AgNPs presented over 80% of cell activity with the expectation of AgNPs-7.

Precisely, by examining the cytotoxicity of AgNPs with the standard of ISO 10993-5, the results (Fig. 4(a)-(c)) established on normal control (blank), negative control and positive control. According to the standard which indicated that the reasonable cell activity is 70% under MTT testing, when the cell activity rate presented nearer or lower than 70% might be considered as possessing toxicity to cells. Under the 24 h incubation for testing cell activity, the outcome (Fig.4(c)) indicated that the experimental groups of AgNPs-1, AgNPs-3 and AgNPs-5 which possessed exceptional cell activity with 93%, 89% and 84% respectively. But, the cell activity of AgNPs-7 (77%) was approaching the critical point of cytotoxicity in the investigation, which might be considered as slightly possessing cytotoxicity for applying on clinical applications. Based on above experimental consequences, AgNPs-5 sample (5 ppm of AgNPs) could be considered as the suitable concentration for potentially against *S. aureus* and still maintained the activity of live cells. Furthermore, AgNPs-5 might be potentially applied as antibacterial agent's concentration for treating bacterial CRS.

3.5 Evaluation of antibacterial effect

On the account of the antibacterial effect results by testing *S. aureus* groups with different AgNPs concentrations (AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7) for 24 h (Fig.5 (a)), the diagram indicated that the antibacterial actions revealed incline gradually with the increase of AgNPs concentration. Notably, 5 ppm and 7 ppm of AgNPs presented over 80% of

antibacterial rate. In addition, for understanding the antibacterial function of AgNPs on *E. coli*, same experiments were examining as well (Fig.5 (b)). The AR result demonstrated 100 % on *E. coli* started with 5 ppm and 7 ppm of AgNPs, which represented that 5 ppm concentration of AgNPs possessed 100% of antibacterial action. By integrating both antibacterial effect testing results on *S. aureus* and *E. coli*, the outcomes indicated that 5 ppm and 7 ppm of AgNPs might possess better antibacterial function on both bacterial strains. And further, based on the prepositive cell activity testing results of AgNPs, the 5 ppm of AgNPs (Fig.4 (b) – 4 (c)) could be considered as better concentration for maintaining over 80% of cell activity rate. Yet, the antibacterial activity between two different types of bacteria would be discussed.

3.6 Antibacterial action on *S. aureus* and *E. coli*

This research investigated the antibacterial action of AgNPs on both Gram-positive *S. aureus* and Gram-negative *E. coli*. The purpose of selecting two types of bacteria was for inspecting the antibacterial function of AgNPs. According to the experimental consequences which indicated that AgNPs might have better antibacterial activity on *E. coli* compared with *S. aureus* in terms of the concentration of 5 ppm AgNPs. Numbers of studies related to microbiology indicated that the most difference between Gram-negative bacteria and Gram-positive bacteria is the thickness of peptidoglycan layer [22]. Gram-negative bacteria possess single peptidoglycan layer [23]; however, Gram-positive bacteria are provided with multiple peptidoglycan layer [24]. Based on this unique feature, which might explain

reasonably the outcomes of this study why 5 ppm of AgNPs possessed 100% of antibacterial effect on Gram-negative *E. coli.* as well as the same concentration of AgNPs had over 80% of antibacterial action on Gram-positive *S. aureus* in 24 h antibacterial experiments.

3.7 Potential application of AgNPs *in vivo*

A review article demonstrated the cell activity comparison of AgNPs and Ag^+ ions [25]. Notably, the results of the review article which matched this research consequence of cell activity that indicated the 5 ppm of AgNPs presented over 80% of cell activity. Other studies revealed the influences of AgNPs by inhalation exposure on rat model [26, 27], both studies indicated that AgNPs might stockpile within olfactory mucosa. The research conducted by Hyun et al. [26] indicated that AgNPs might potentially impact the neutral mucins within the respiratory mucosa; yet, no evidence of toxicology presented statistically significance. While, Oberdörster et al. [27] pointed out that ultrafine nanoparticles might potentially transport into brain to cause certain damages on account of his research outcome. Many studies presented that the biocompatibility of AgNPs might highly relate to its exposure dose *in vivo* [25, 28-30]. As mentioned above, these research results demonstrated that 5 ppm of AgNPs might possess effective antibacterial activity both on *S. aureus* and *E. coli* bacteria, and still maintaining functions of live cells. Additionally, no other similar studies with this research were discovered, especially on defining the suitable concentrations of AgNPs for better biocompatibility and effective antibacterial capability for CRS applications. However, more

tests should be conducted to demonstrate that the AgNPs with concentration of 5 ppm and diameter of 10 nm could be considered as a promising treatment method for CRS applications in the future.

4. Conclusion

AgNPs of 5 ppm had better biocompatibility on RPMI2650 cells and effective antibacterial function both on *S. aureus* and *E. coli* according to experimental consequences of cell cytotoxicity assay and antibacterial effect assay. Above on those facts which might give a direction toward a potential future treatment that AgNPs could be considered to apply on treating CRS. However, for increasing the capability of antibacterial function at the certain concentration of 5 ppm of AgNPs, further research will focus on reinforcing the cell activity, conductions of AgNPs into cells, and further, establishing certain animal models for investigating biocompatibility and effective antibacterial capability of AgNPs *in vivo*.

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References

[1] J.B. Shi, Q.L. Fu, H. Zhang, L. Cheng, Y.J. Wang, D.D. Zhu, W. Lv, S.X. Liu, P.Z. Li, C.Q. Ou, G. Xu, Allergy, 70 (2015) 533-539.

[2] S.C. Payne, M.S. Benninger, Clinical Infectious Diseases, 45 (2007) e121-e127.

[3] P. Eloy, A.L. Poirrier, C. De Dorlodot, T. Van Zele, J.B. Watelet, B. Bertrand, Current Allergy and Asthma Reports, 11 (2011) 146-162.

[4] A. Niederfuhr, H. Kirsche, T. Deutschle, S. Poppert, H. Riechelmann, N. Wellinghausen, Allergy, 63 (2008) 1359-1367.

[5] M.E. Kreft, U.D. Jerman, E. Lasič, T. Lanišnik Rižner, N. Hevir-Kene, L. Peternel, K. Kristan, Pharmaceutical Research, 32 (2015) 665-679.

[6] A. Panáček, M. Smékalová, M. Kilianová, R. Prucek, K. Bogdanová, R. Večeřová, M. Kolář, M. Havrdová, G. Płaza, J. Chojniak, R. Zbořil, L. Kvítek, Molecules, 21 (2016) 26.

[7] Sinus, A.H. Partnership, Otolaryngology -- Head and Neck Surgery, 130 (2004) 1-45.

[8] Spector SL, Bernstein IL, Li JT, Berger WE, Kaliner MA, Schuller DE, Blessing-Moore J, Dykewicz MS, Fineman S, Lee RE, N. RA., J Allergy Clin Immunol. , 6 Pt2 (1998) S107-144.

[9] G. Gahlawat, S. Shikha, B.S. Chaddha, S.R. Chaudhuri, S. Mayilraj, A.R. Choudhury, Microbial Cell Factories, 15 (2016) 1-14.

[10] P.N. Lim, L. Chang, E.S. Thian, Nanomedicine: Nanotechnology, Biology and Medicine, 11 (2015) 1331-1344.

[11] C.-F. Huang, H.-J. Chiang, W.-C. Lan, H.-H. Chou, K.-L. Ou, C.-H. Yu, Biofouling, 27 (2011) 449-457.

[12] S.F. Ou, R.J. Chung, L.H. Lin, Y.C. Chiang, C.F. Huang, K.L. Ou, J Alloy Compd, 629 (2015) 362-367.

[13] S. Prabhu, E.K. Poulose, International Nano Letters, 2 (2012) 1-10.

[14] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez, M.J. Yacaman, Nanotechnology, 16 (2005) 2346-2353.

[15] M.J. Yacaman, J.A. Ascencio, H.B. Liu, J. Gardea-Torresdey, J Vac Sci Technol B, 19 (2001) 1091-1103.

[16] D.W. Hatchett, H.S. White, J Phys Chem-Us, 100 (1996) 9854-9859.

[17] N. Doraiswamy, L.D. Marks, Surf Sci, 348 (1996) L67-L69.

[18] F. Raimondi, G.G. Scherer, R. Kotz, A. Wokaun, Angew Chem Int Edit, 44 (2005) 2190-2209.

[19] D.W. Zhang, D.D. Liub, J.C. Zhang, C.C. Fong, M.S. Yang, Mat Sci Eng C-Mater, 49 (2015) 871-871.

[20] X. Liu, N. Huang, H. Li, Q. Jin, J. Ji, Langmuir, 29 (2013) 9138-9148.

[21] N. Oh, J.H. Park, Int J Nanomedicine, 9 Suppl 1 (2014) 51-63.

[22] E. Hoiczyk, A. Hansel, Journal of Bacteriology, 182 (2000) 1191-1199.

[23] T.J. Beveridge, Journal of Bacteriology, 181 (1999) 4725-4733.

[24] O. Schneewind, D.M. Missiakas, Philosophical Transactions of the Royal Society of London B: Biological Sciences, 367 (2012) 1123-1139.

[25] T. Zhang, L. Wang, Q. Chen, C. Chen, Yonsei Med J, 55 (2014) 283-291.

[26] J.-S. Hyun, B.S. Lee, H.Y. Ryu, J.H. Sung, K.H. Chung, I.J. Yu, Toxicology Letters, 182 (2008) 24-28.

[27] G. Oberdörster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox, Inhalation Toxicology, 16 (2004) 437-445.

[28] T.M. Scown, E.M. Santos, B.D. Johnston, B. Gaiser, M. Baalousha, S. Mitov, J.R. Lead, V. Stone, T.F. Fernandes, M. Jepson, R. van Aerle, C.R. Tyler, Toxicological Sciences, 115 (2010) 521-534.

[29] K.J. Lee, P.D. Nallathamby, L.M. Browning, C.J. Osgood, X.-H.N. Xu, ACS Nano, 1 (2007) 133-143.

[30] K.H. Wu, Y.C. Chang, W.Y. Tsai, M.Y. Huang, C.C. Yang, Polymer Degradation and Stability, 95 (2010) 2328-2333.

Figure captions

Fig. 1 (a) bright field electron micrograph of the [011] zone of the original AgNPs solution sample and (b) EDS spectrum taken from the black spherical particle in (a) (The signals of Cu resulted from the Cu grid).

Fig. 2 (a) TEM image of the RPMI2650 cell without adding AgNPs as control. (b) TEM image of the RPMI2650 cell after adding AgNPs. The phenomenon of AgNPs entering the RPMI2650 cell was observed.

Fig. 3 For tracking *S. aureus* before and after entering the RPMI2650 cell by fluorescence of *S. aureus*, and further, observing the changes of cell morphology after adding bacteria for 1 h. (a) image of RPMI2650 cell without adding *S. aureus* under OM. (b) image of RPMI2650 cell without adding *S. aureus* under FM. (c) image of the RPMI2650 cell after adding *S. aureus* for 1 h under OM. (d) image of RPMI2650 cell after adding *S. aureus* for 1 h under FM.

Fig. 4 (a) the change of cell morphology after adding different concentrations of AgNPs medium (AgNPs-1, AgNPs-3, AgNPs-5 and AgNPs-7) into RPMI2650 cells for 24 h then observed by OM. (b) MTT testing results with 24 h incubation under λ = 570 nm. (c) MTT testing results of cell activity with 24 h incubation. (**P* value < 0.05; ***P* value < 0.005)

Fig. 5 (a) antibacterial effect testing on *S. aureus* under 37°C, 24 h incubation, 5 ppm of AgNPs revealed over 80% of antibacterial rate and (b) antibacterial effect testing on *E. coli* under 37°C, 24 h incubation, 5 ppm and 7 ppm of AgNPs revealed 100% of AR.

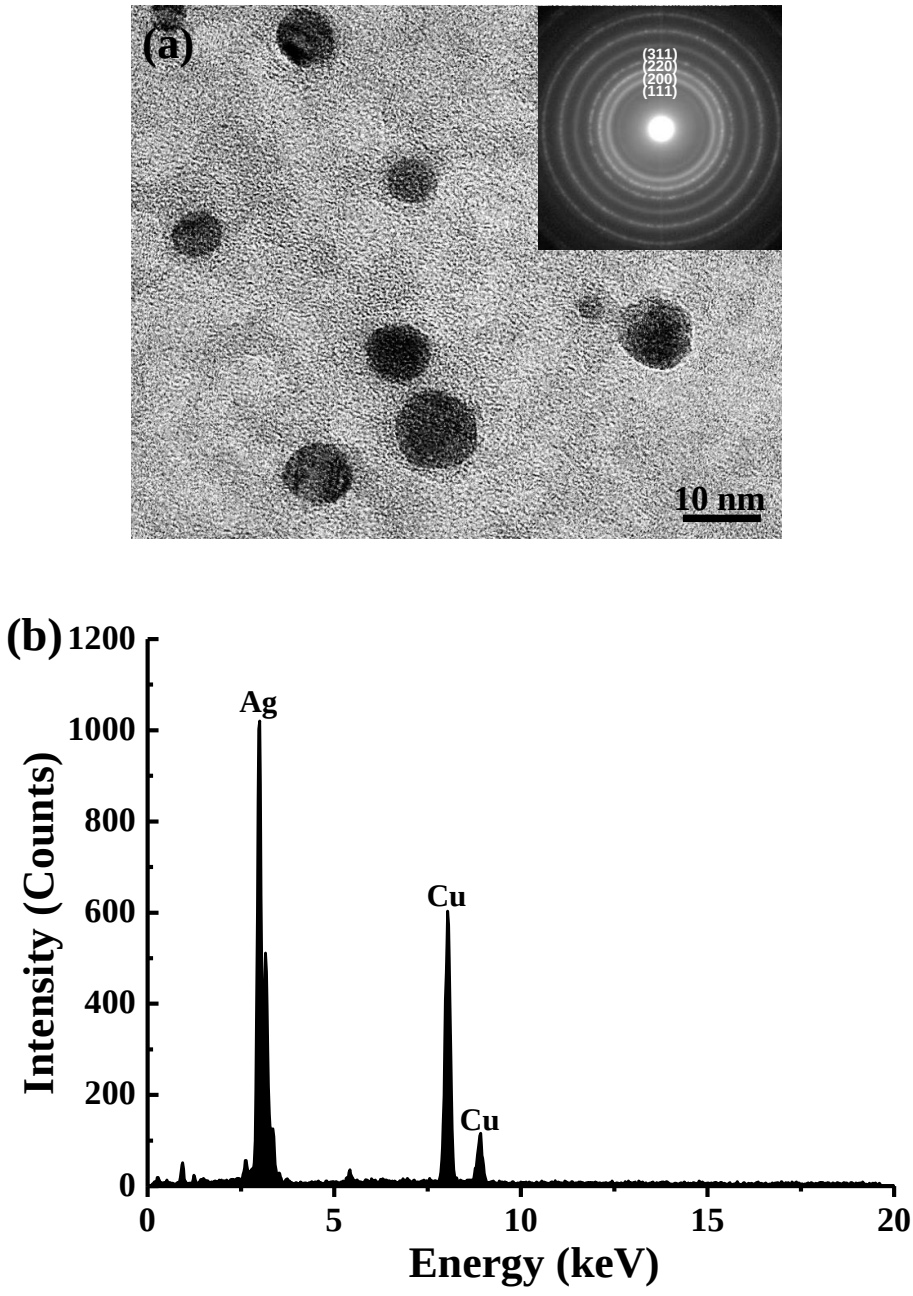

Figure 1

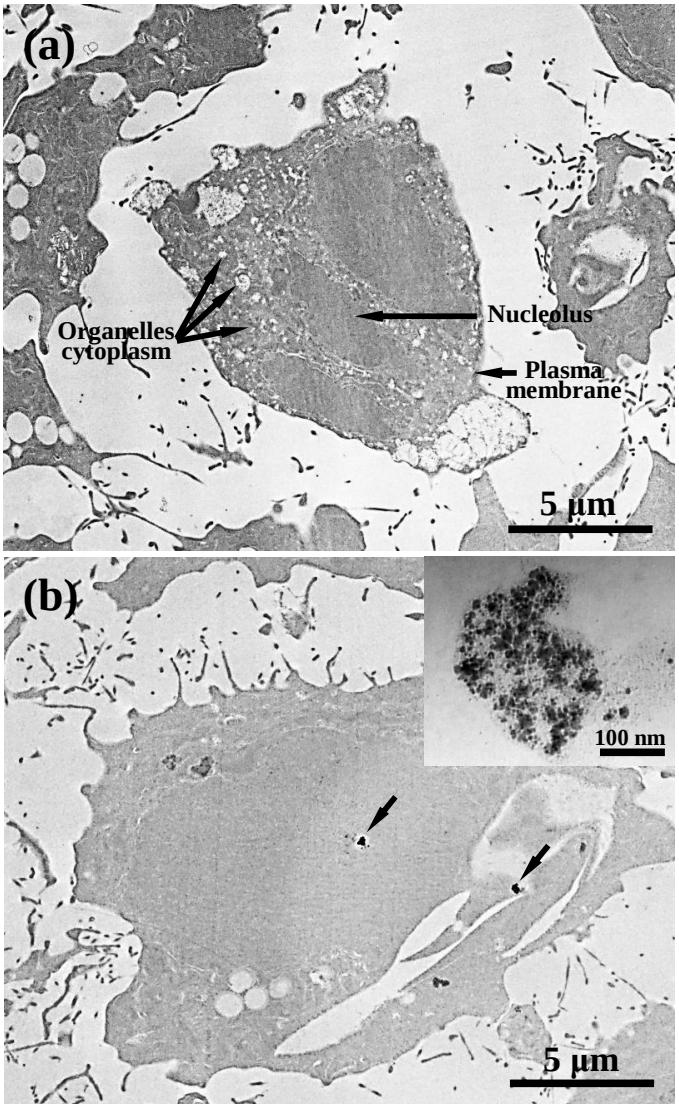

Figure 2

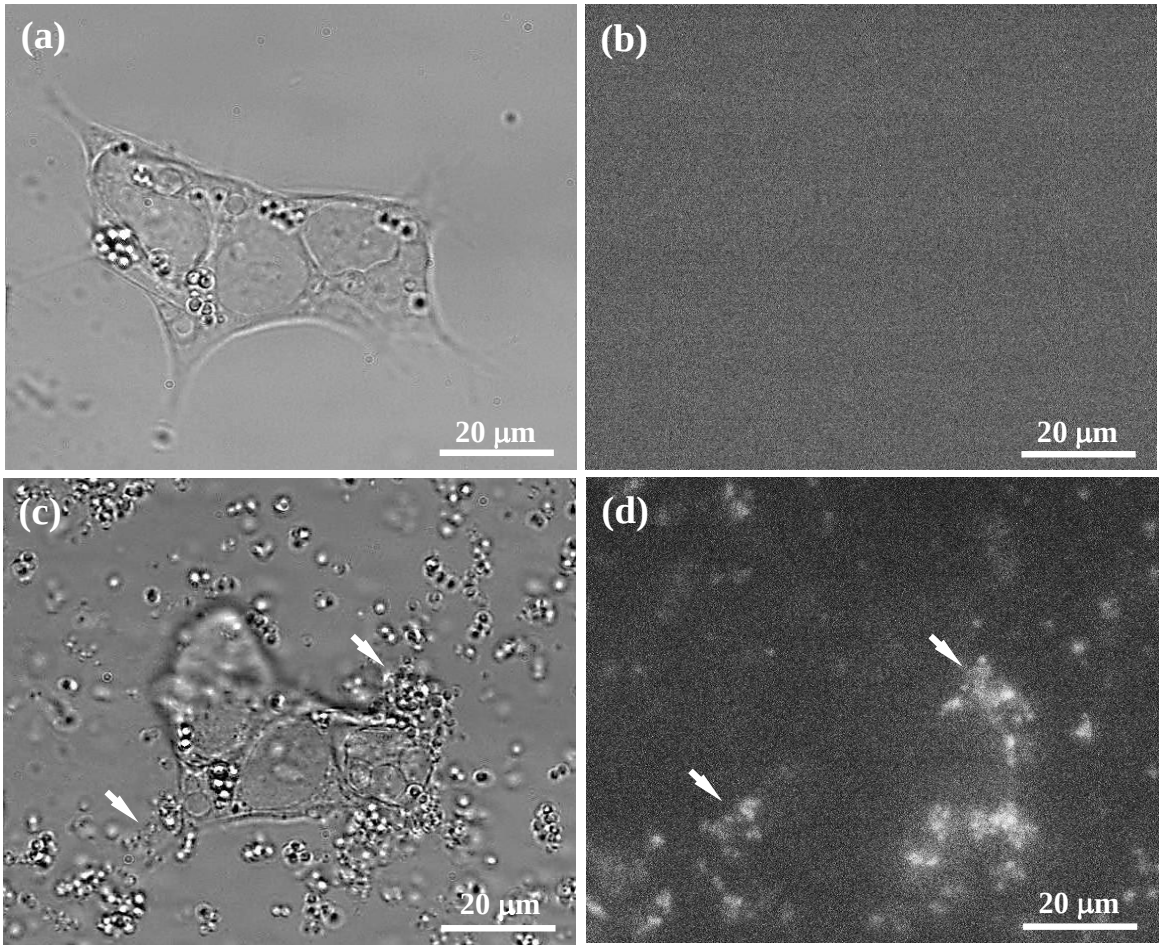

Figure 3

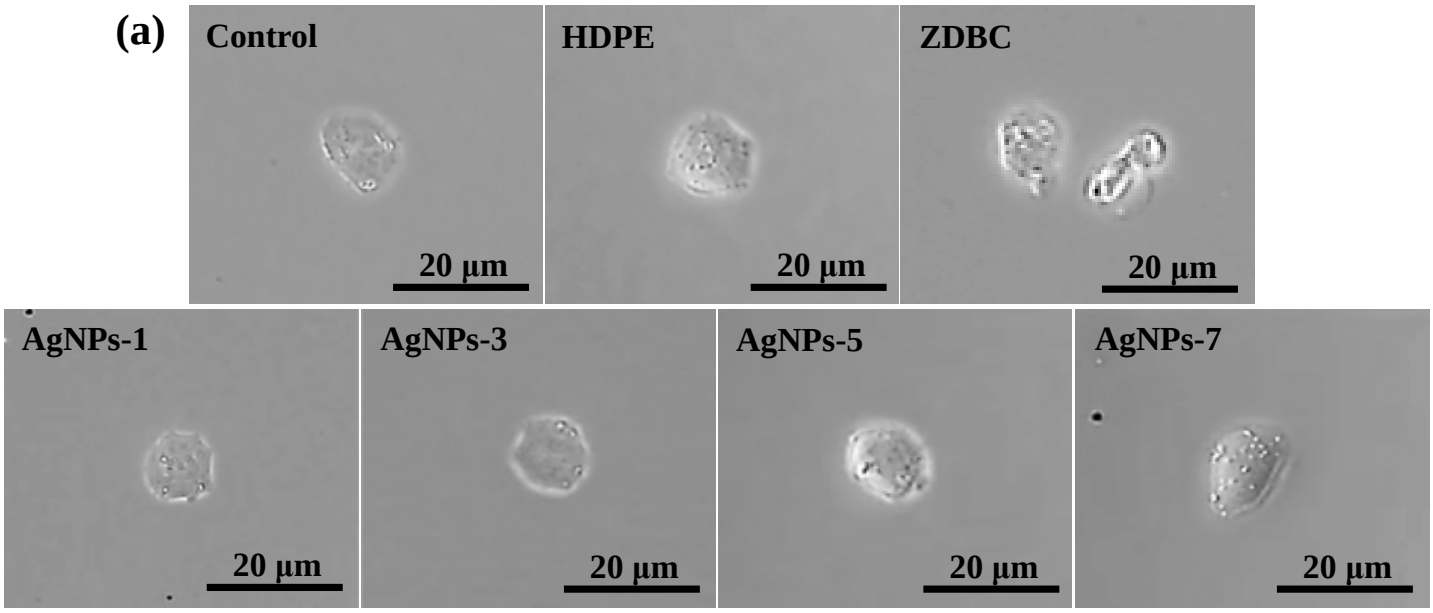

Figure 4 (a)

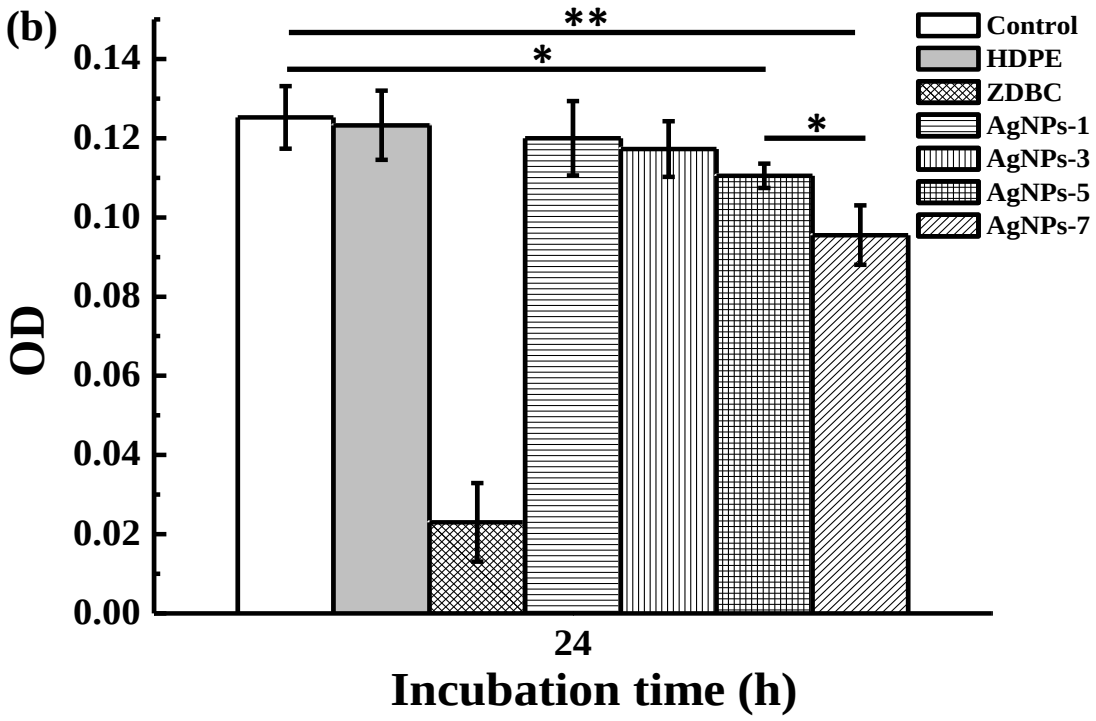

Figure 4 (b)

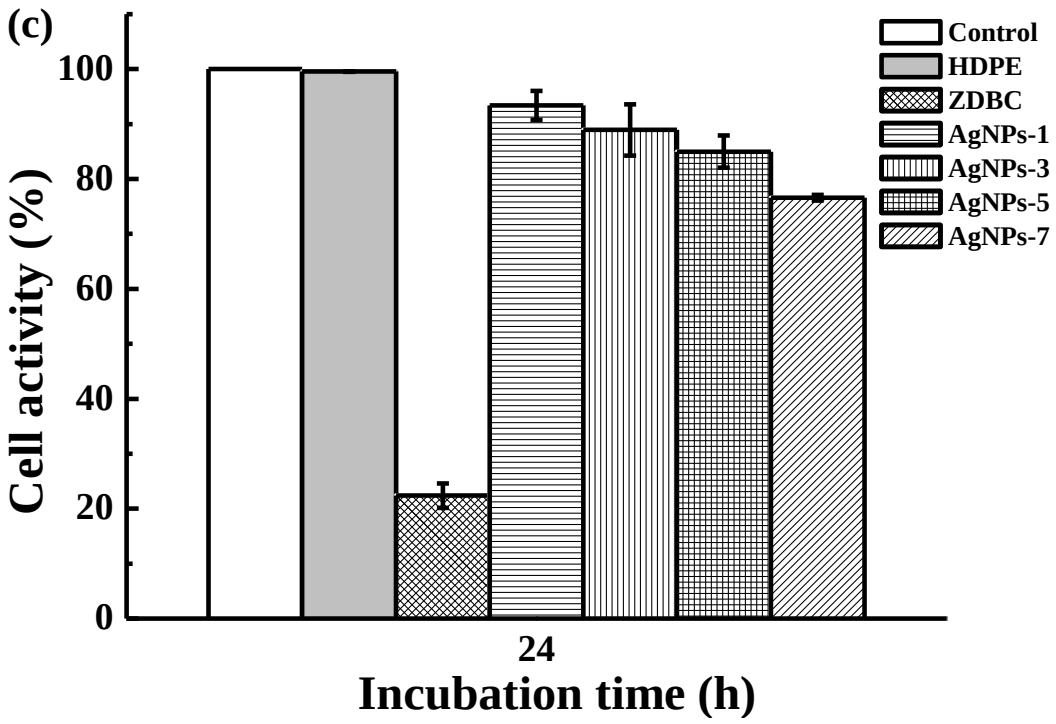

Figure 4 (c)

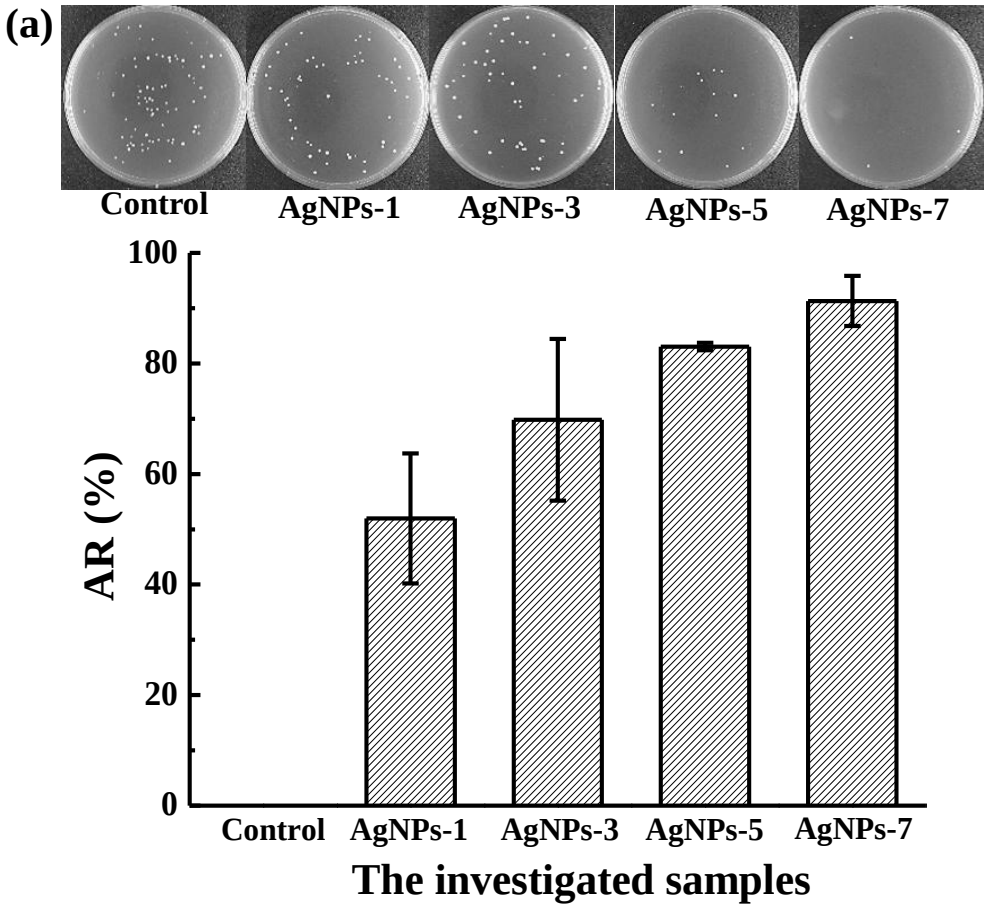

Figure 5 (a)

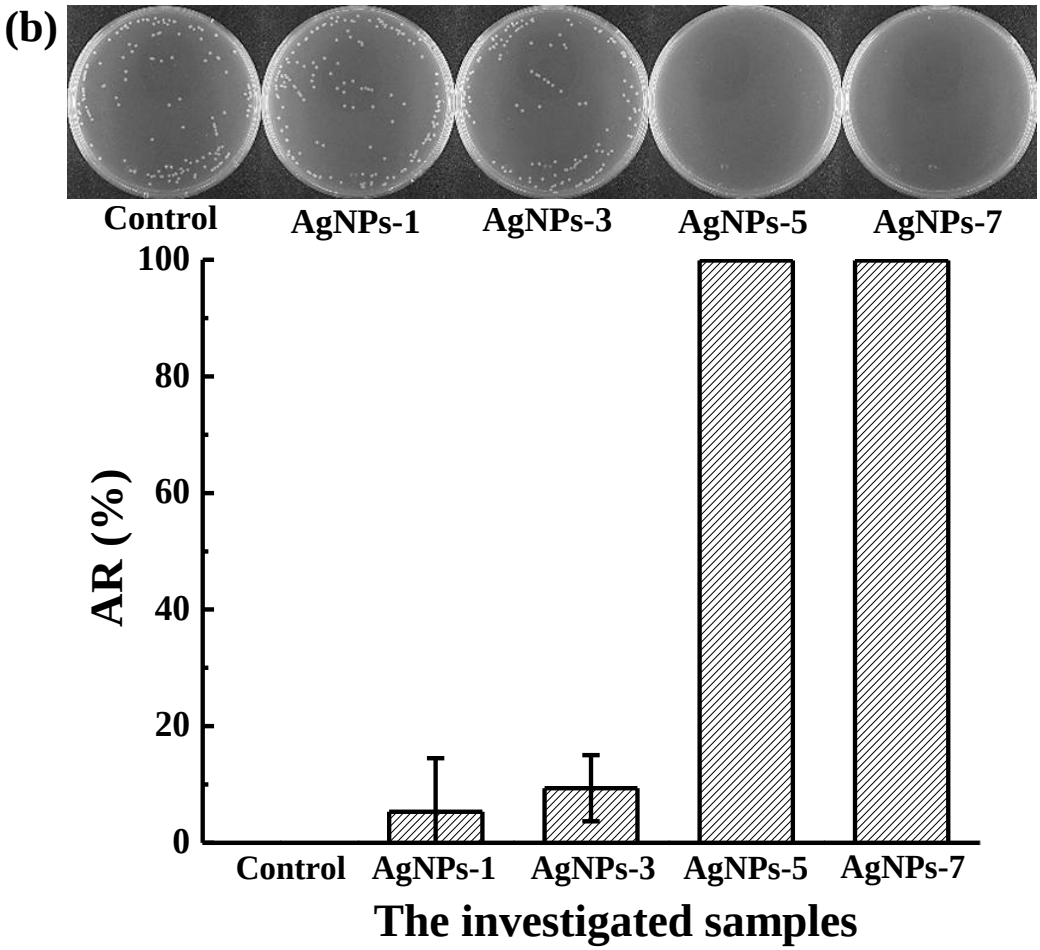

Figure 5 (b)

Research highlights

- The 10 nm silver nano-particles (AgNPs) could penetrate cell membrane into RPMI2650 cell.
- The 5 ppm of AgNPs had better biocompatibility on RPMI2650 cells.
- The 5 ppm of AgNPs possessed 80% of antibacterial effect on *S. aureus*.
- The 5 ppm of AgNPs is a promising antibacterial agent for treating chronic rhinosinusitis.