Cumhuriyet Üniversitesi Fen Fakültesi
Fen Bilimleri Dergisi (CFD), Cilt:36, No: 3 Özel Sayı (2015)
ISSN: 1300-1949

Cumhuriyet University Faculty of Science
Science Journal (CSJ), Vol. 36, No: 3 Special Issue (2015)
ISSN: 1300-1949

Comparing the antimicrobial effects of silver and copper nanoparticles against pathogenic and resistant bacteria of Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus

Yasaman Sadat NABIPOUR[1,*], Arman ROSTAMZAD[2]

[1]*MSc, Dept of Biology, Ilam University, Ilam, Iran*

[2]*Assistant Professor, Dept of Biology, Ilam University, Ilam, Iran*

Received: 01.02.2015; Accepted: 05.05.2015

Abstract. Topics and Objectives: In this study, the effectiveness of antimicrobial activity of Ag and Cu nanoparticles against Gram positive bacteria and Gram-negative resistant pathogens and common nosocomial infections were observed.Methods: At first, in order for the effect of different concentrations of nanoparticles on bacteria in macrodilution method of 0.01, 0.1, 0.5, 1, 1.5 % of silver and copper nanoparticles (medium + nanoparticles) were prepared and added to 105 cell/ml of each of the bacteria.Dishes containing the treated cultures (bacteria + nanoparticles) and control media were placed in a shaking incubator. Then the OD of the treatment and control cultures + control were determined.Achievements of the paper: In statistical analysis, it was revealed that about nanoparticles of Ag and Cu, 0.5 percent concentration of bactericidal particles are able to remove almost 100% of Klebsiella pneumoniae bacteria, Pseudomonas aeruginosa, Staphylococcus aureus and 0.1 percent concentration of each of the 3 bacteria was bacteriostatic.

Keywords: nanoparticle, macrodilution, bacteriostatic, bactericidal

1. INTRODUCTION

The belief that nanotechnology is another era of science and is an integration of engineering and biology, chemistry, medicine and physics, is accepted by most scientists [1]. Studies have shown that the smaller the nanoparticles, they show different and new characteristics and activities. These features makes the use of nanomaterials to spread rapidly so that in all aspects of life, such as electrical systems, they are against microbes [2]. Metal nanoparticles are used in insecticides and bactericidal for years [3]. Some nanoparticles are regarded as a novel approach to the development of modern pharmaceutical science which are frequently used in biology and pharmacology studies due to the high potential to perform specific treatment processes. For example, they are able to destroy 650 cancer cells in less than 4 hours [4].

Nanoparticles have illustrated the lowest level of toxicity in the life cycle and ecosystem, therefore, the use of these substances to fight against the pathogens can be a proper choice [5]. Metal nanoparticles, on the basis of surface to volume ratio, show different anti-bacterial characteristic. Gram-positive bacteria in comparison to gram-negative bacteria against metal nanoparticles, indicate more resistance that can be related to the structure of the cell wall [6].

2. MATERIALS AND METHODS

In this study, first the nanoparticles were prepared and the effect of different concentrations of [0.01, 0.1, 0.5, 1, 1.5 %] of the pathogens were studied.

*Corresponding author. *Email address: y.nabipour1@gmail.com*

Special Issue: The Second National Conference on Applied Research in Science and Technology

The studied Nanoparticles were bought from Nanoshell company of America. To check the sizing of nanoparticles, TEM images were taken with an Electron Microscope of Philips, Model H987, to determine the size of the nanoparticles. For distributing the nanoparticles in culture medium, nanoparticles Solution were placed in Cu Shaker before being used, and after inoculation, the culture was carried out in a shaking incubator. Also the amount of inoculated nanoparticles was not enough to precipitate or not having uniform distribution.

Diameter of the nanoparticles used in this study Ag: 20nm, and Cu: 20nm the complex medium of (Complex) LB Lauria Bertani) was used for the growth and storage medium of bacteria.

three strains were used in this study:

1.Staphylococcus Aureus (ATCC 29213)

2.Klebsiella pneumoniae (ATCC 7881)

3.Pseudomonas aeruginosa (ATCC 31480)

The above strains of scientific research and industrial organizations were prepared by live freezing.

3. THE EFFECT OF DIFFERENT CONCENTRATIONS OF NANOPARTICLES ON BACTERIA IN MACRO DILUTION ENVIRONMENT

At first liquid broth (LB) was synthesized and sterilized, after that, the nanoparticles were weighed by electronic weighing scales by the weights of 0/001 g, 0/01 g, 0/05 g, g 0/10, 0/15 g, and by adding these particles to 10 ml of liquid broth (LB), the concentrations of 0/01 percent, 0/1 percent, 0/5 percent, 1 percent and 1/5 percent of nanoparticles Ag, Cu, were prepared as suspension.

Again, to sterilize, the broth was placed in the automatic autoclave by the temperature of 121 ° C for 15 min and a pressure of 15 pounds (Lb15) and then was kept in the refrigerator for cooling. To prepare a new culture of Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa bacteria of Mueller-Hinton agar medium (Merck), 34 grams in one liter of distilled water was used [7].

Then to prepare bacterial suspension, one loop of each strain of bacteria was added into 25 ml of liquid broth (LB), at the next level by SPECT device, concentration containing of optical density of (OD) 0/05 at the wavelength of 600 nm was prepared, to obtain a final concentration of 10^5 cell / ml per ml of each of the samples.

At the next level, by Sampler 2 ml, the concentration prepared from each bacteria, were added to the concentrations prepared from (liquid broth + nanoparticles) and the same procedure was used to prepare positive control group with the exception that the nanoparticles were not added to the liquid broth. the liquid broth without bacteria was used as negative control. The Lid of the containers containing the treated liquid broth (bacteria + nanoparticles) and the closed control Liquid broth media and in an incubator shaker at 250 rpm at 37 ° C for 24 h, were cultured.

After the mentioned period, the optical density at a wavelength of 600 nm was used for measuring the concentration of the bacteria, and the treated and positive control and negative control respectively poured in glass Couettes and the OD was determined. The concentration of nanoparticles in the bacteria culture medium was used as a Blanc solution to the calibration of the spectrophotometer. This experiment was repeated three times and the mean values were reported. Photographs were taken by a digital camera Olympus C2020Z. In all tests, the results obtained were compared with the control group. SAS software and Student t-test test was used to determine the significance (P-value <0/01) of their results and evaluation.

4. FINDINGS

4.1. The effects of nanoparticles (Cu) on the studied bacteria at LB medium:

According to the statistical analysis of the optical density from the SPECT of the treated medium, it was found that the concentration of 0/01 percent of Cu nanoparticles did not have significant antibacterial effect on the studied bacteria, however, concentrations of 0/1% and 0/5%, had a large inhibitory effect on the growth of bacteria in the treated groups compared with the control group. At the concentration of 0/5% of Cu nanoparticle of all three bacteria were bactericidal and more than 80% of the bacteria have gone away. Similarly, with increasing of the concentrations of Cu nanoparticles, the bactericidal effect increases. By concentration of 0/5% Cu nanoparticles fatality rate compared with the control group in the Klebsiella bacteria has been 91% and in the Staphylococcus aureus bacteria has been 72%. At this concentration, the Cu nanoparticles had the largest effect on Pseudomonas bacteria so that compared to the control group, this bacteria shows 94% reduction in the growth. Also Cu nanoparticles at the concentrations of 0/1% has been able to remove 76% of Klebsiella bacteria, 69% of staph bacteria and 83% of Pseudomonas bacteria. In total Cu nanoparticles had the largest effect on Pseudomonas bacteria So that at the concentration of 0/1% the bacteria has 83% reduction in the growth, and at the concentration of 0/5% it shows 94% reduction in growth. Also the effect of Cu nanoparticles on the Pseudomonas bacteria at the concentration of 1% had the bactericidal effect to 98%. This result was slightly different from the results of Adams et al. so that according to their study, the growth inhibitory concentration obtained in 48% of Pseudomonas species at the concentration of 1000 mgL of nanoparticle Cu, [8].

In this case, the main reason for the difference in two studies can be the size of the nanoparticles and the culture and strain differences so that the size of the nanoparticles is found to be effective in their toxicity [9].

Minimal impact of Cu nanoparticles was seen on the Staphylococcus aureus bacteria so that the bacteria at the concentrations of 0/1 % of Cu had 69% reduction in growth that compared to other Bacteria shows the minimum sensitivity and maximum resistance. Also, the bacteria at the concentration of 0/5% of nanoparticles Cu, showed 72% reduction in growth compared to the positive control group that compared to the concentration of 0/1 % did not have much differences in mortality of bacteria. However, the concentration of 1% of Cu on the bacteria was effective and the bactericidal effect was high and compared to the control group, 92% of the bacteria are gone. The results of the determination of antibacterial nature of nanoparticles showed a direct correlation between the concentration of nanoparticles and bacteria removal rates.

These results are statistically significant (P-value< 0/01), on the basis of the obtained data, it was found that removal of the studied bacteria by various nanoparticles, is different. (Table 1) and (Figure 1)

Table 1. Changes in optical density of the studied bacteria, from the effect of different concentrations of Cu nanoparticles at the LB medium

Cu	C	0.01	0.1	0.5	1	1.5
K	1.115	1.104	0.275	0.065	0.034	0.009
S	1.203	1.150	0.376	0.092	0.047	0.099
p	1.250	1.010	0.204	0.043	0.028	0.007

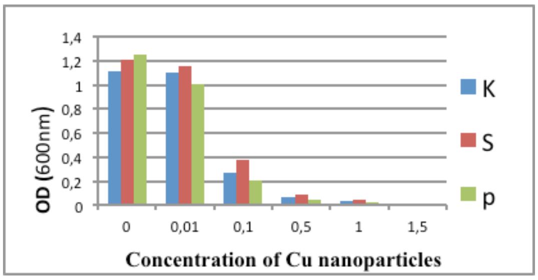

Figure 1. Effect of different concentrations of Cu nanoparticles on pathogenic Klebsiella pneumoniae (K), Staphylococcus aureus (S), Pseudomonas bacteria (p) at LB medium.

4.2. The effects of nanoparticles (Ag) on the studied bacteria at LB medium:

According to the statistical analysis of the optical density from the SPECT of the treated medium, it was found that the concentrationof 0/01 percent of Cu nanoparticles did not have significant antibacterial effect on the studied bacteria, however, concentrations of 0/1% and 0/5%, had a large inhibitory effect on the growth of bacteria in the treated groups compared with the control group. At the concentration of 0/5% the bactericidal rate of Ag nanoparticle was strong and reaches almost 100%. 0/1%concentration of these nanoparticles bacteriostatic are bacteriostatic. The nanoparticles at concentrations of 0/1% have the most inhibitory effect on Pseudomonas aeruginosa and have destroyed These bacteria at the rate of 92%. Ag nanoparticles had the minimal effect on Staphylococcus aureus bacteria so that at the concentration of 0/1% of nanoparticles Ag, this bacteria showed 72% reduction in the growth. The mechanism of nanoparticles Ag is in this way that Cellular respiration in the presence of various concentrations of Ag nanoparticles in different bacterial groups presents a similar pattern and that is the gradual reduction of cellular respiration is parallel with the increasing concentration of Ag nanoparticles, so that at the concentration of MIC for each bacterial, respiration rate is the lowest [10]. The results of the determination of antibacterial nature of Ag nanoparticles showed a direct correlation between the concentration of nanoparticles and bacteria removal rates(Table 2), (Figure 2). By increasing the concentration of nanoparticles, bactericidal activity was added, which is in correspondce with previous studies. These results are statistically significant (P-value< 0/01)

Table 2. Changes in optical density of the studied bacteria, from the effect of different concentrations of Ag nanoparticles at the LB medium.

Ag	C	0.01	0.1	0.5	1	1.5
K	1.438	1.216	0.298	0.015	0.005	0.002
S	1.427	1.360	0/399	0.041	0.028	0.017
p	1.496	1.117	0.107	0.019	0.008	0.005

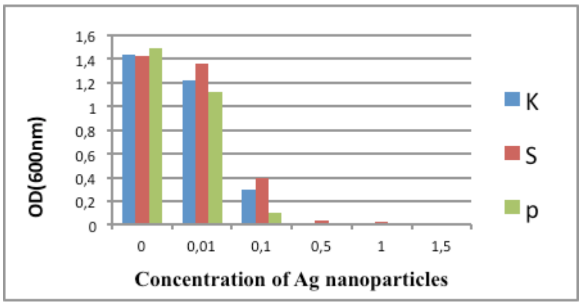

Figure 2. Effect of different concentrations of Ag nanoparticles on pathogenic Klebsiella pneumoniae (K), Staphylococcus aureus (S), Pseudomonas bacteria (p) at LB medium.

In the present study, we evaluated the antibacterial effects of Ag and Cu on gram-positive and gram-negative bacteria, which are resistant to nosocomial infections. The findings of the present study agree with the previous studies, which are dealing with the antibacterial effects of nanomaterials. The nanoparticles lead to the peroxidation of the phospholipid multi-ring compounds of the membrane Lipid of bacteria and therefore the integrity of the cell membrane reduces, and normal cellular activities in a healthy cell structure such as the respiratory activities disappear and cell death becomes unavoidable. Norns et al. in 2010 in a research entitled " Colloidal Silver as a New Antimicrobial Agent " reported that antibacterial activity of Colloidal silver has superior effect against Escherichia coli and Salmonella typhimurium compared with other antibiotics [11]. The results of the antibacterial effect of silver nanoparticles in the Norns study is consistent with this study.

Shrivastava et al. in 2010 examined Nanosilver antibacterial effect on Staphylococcus aureus and Salmonella Typhimurium and showed that Nanosilver antibacterial effect of the particles is dependent on the dose, and these are more effective against gram-negative bacteria than Gram-positive bacteria [12], which is in correspondce with the results of this research.

Hvtay Lee et al. In 2012, in a study entitled "Evaluation of antibacterial polyethylene pipe with high-density of nanosilver coating " concluded that HDPE -Ag Pipes have a potential anti-bacterial ability [13], which corresponded with the results of this research.

Dutta R.K in 2012 in their study entitled Synthesis and evaluation of potential antibacterial properties of zinc oxide, determined that the Antibacterial nature of the silver nanoparticles is influenced by its concentration [14], which is in correspondce with the results of this research.

In the study of Selvam et al. in 2012 it was found that the rate of growth inhibitory concentrations of bacteria varies depending on the type of bacteria [15], which is in correspondce with the results of this research.

Matthews et al. In 2010, in a study entitled "Application of nano antibacterial drugs in the diagnosis and medical treatment", stated that Ag nanometer scale can be used to treat and completely inhibits the growth of high percentage of Gram-positive and Gram-negative bacterial species [16], which is in correspondce with the results of this research.

Hvmbrtv et al. In 2010, evaluated the inhibitory effects of silver nanoparticles on the bacteria that show big drug resistance, and observed that Ag nanoparticles have significant bacteriostatic effect on these bacteria [17], which is in correspondce with the results of this research.

Yin Guang Li et al. In 2007, in a study entitled "Synthesis of nano-silver colloids and their antimicrobial effect" declared that large parts of the bacteria were destroyed by treatment by Ag nanoparticles, in addition, with low concentrations of Ag nanoparticles, growth inhibition is created that results in a significant reduction in the amount of live bacteria compared to the control sample [18], which is in correspondce with the results of this research.

Possible reaction mechanisms and the interaction of nanomaterials with biological macromolecules is in this way that nano materials release ions that react with proteins on the surface of bacterial cells (-SH) Thiel. These proteins have bulge from the membrane of bacterial cells, and result in the transfer of nutrients from the cell walls. nanomaterials disable these proteins, reduce the membrane permeability and eventually lead to cell death [19].

5. CONCLUSION

In total, the results of the present study prove a number of issues:

1. The metallic nanoparticles have very effective antibacterial properties.
2. The effect of colloidal nanoparticles, is dependent on concentration.
3. The effect of colloidal nanoparticles in the mentioned bacteria is in the form of fatality not inhibitory, so that in all bacteria MIC is equal to MBC.
4. In the present study Staphylococcus aureus were the most resistant strains, and Vashrshya coli were the most sensitive strains, compared to the bactericidal properties of the nanoparticles.
5. The solution of colloidal nanoparticles release in the form of microscopic particles and can easily penetrate into the cells of bacteria.
6. Ag and Cu nanoparticles, which are made based on the biological effects of nanoparticles, can be used to treat infections and diseases created by S. aureus and p. Aeruginosa and K. pneumoniae.

REFERENCES

[1] Li, P; Li, J; Wu, C; Wu, Q; "Synergistic antibacterial effects of Lactam antibiotic combined with silver nanoparticles", J Nanotechnol; 16:1912-1917(2005).
[2] Dowling, A; Clift, R; Grobert, N; Hutton, D; Oliver, R; O'Neill, O, et al; Nanoscience and Nanotechnologies: Opportunities and Uncertainties. London UK Royal Society and Royal Academy of Engineering, 211: 1242-50(2007).
[3] Tai-Lee, Hu, Anti-bacterial study using nano silver-doped high density polyethylene pipe. Sustain. Environ. Res; 22(3), 153-158 (2012).
[4] Kumar, A; Jakhmola, A; "RNA-mediated fluorescent Q-Pb nanoparticles", Langmuir, 23:2915-2918(2007).
[5] Sun, Y G; Mayers, B; Herricks, T; Xia, Y N; "Polyol synthesis of uniform silver nanowires: a plausible growth mechanism and the supporting evidence". J Nano Lett; 3:955-960(2003).
[6] Te-Hsing, W; Yi-Der, T; Lie-Hang, S; "The novel methods for preparing antibacterial fabric composites containing nanomaterial". J Solid State Phenomena, 2007; 124:1241-1244.
[7] Te-Hsing, W; Yi-Der, T; Lie-Hang, S; "The novel methods for preparing antibacterial fabric composites containing nano-material", Solid State Phenomena, 124-126: 1241-4(2007).
[8] Shrivastava, S; jyung, woo; lungue, M; Characterization of Enhanced Antibacterial Effects of Nano Silver Nano Particles, J Nanotechnology, 25(12):103– 125(2010).
[9] Wen-Ru, Li, Antibacterial activity and mechanism of silver nanoparticleson Escherichia coli ; Appl Microbiol Biotechnol (2010).

[10] Shrivastava, S; jyung, woo; lungue, M; Characterization of Enhanced Antibacterial Effects of Nano Silver Nano Particles, J Nanotechnology, 25(12):103– 125(2010).
[11] Amanda, S; Mohammad, F; John, J; Schlager, D; Syed, A;"Metal-based nanoparticles and their toxicity assessment", J Nanomed Nanobiotechnol, 2:544-568(2010).
[12] Lara, HH; Ayala-Núnez, NV; Ixtepan Turrent, LC; Rodríguez Padilla, C;Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World Journal of Microbiology and Biotechnology, 26(4):615-21(2010).
[13] Guangyin, Lei;Synthesis of Nano-Silver Colloids and Their Anti Microbial Effects Master of Science In Materials Science & Engineering. Blacksburg, Virginia, November 8(2007).
[14] Tai-Lee, Hu, Anti-bacterial study using nano silver-doped high densitypolyethylene pipe. Sustain. Environ. Res. 22(3), 153-158 (2012).
[15] Dutta, R. K, Et Al. "Studies On Antibacterial Activity Of Zno Nanoparticles By ROS Induced Lipid Peroxidation", Colloids Surf.B Biointerfaces. 94:143-50(2012).
[16] Selvam, S, Et Al. "Antibacterial Effect Of Novel Synthesized Sulfated Beta-Cyclodextrin Crosslinked Cotton Fabric And Its Improved Antibacterial Activities With Zno, Tio(2) And Ag Nanoparticles Coating." Int.J.Pharm. 434.1-2 : 366-74(2012).
[17] Liam, Matthews, Applications of Nanomedicine in Antibacterial Medical Therapeutics and Diagnostics. The Open Tropical Medicine Journal, Volume 3(2010).
[18] Guangyin, Lei, Synthesis of Nano-Silver Colloids and Their Anti Microbial Effects Master of Science in Materials Science & Engineering. Blacksburg, Virginia, November 8(2007).
[19] Lara, HH; Ayala-Núnez, NV; Ixtepan Turrent, LC; Rodríguez Padilla, C, Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World Journal of Microbiology and Biotechnology, 26(4):615-21(2010).