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Evaluation of Antimicrobial Activity of Zinc Oxide Nanoparticles Against Human Pathogens

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Abstract

Introduction: The purpose of this study was to evaluate the antimicrobial effects of zinc oxide nanoparticles on human bacteria.

Methods: The commercially available zinc oxide nanoparticles were purchased. The studied bacteria were purchased as lipophilic ampoules from Iranian Fungal and Bacterial Collection. Finally, the minimum inhibitory concentration and minimum bactericidal concentration were determined by the microdilution method.

Results: The results of this study showed that the maximum inhibitory concentration of zinc oxide nanoparticles was 1500 µg/mL against *Staphylococcus aureus* and *Streptococcus pyogenes*. While the minimum inhibitory concentration was 187 µg/mL against *Acinetobacter baumannii*. The maximum bactericidal concentration (3000 µg/mL) was observed against *S. aureus*. While the minimum bactericidal concentration was observed against Acinetobacter baumannii.

Conclusion: Based on our results, zinc oxide nanoparticles have a good antimicrobial activity against bacteria.

Keyword: Zinc oxide, Antibacterial, Human pathogen

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Introduction

Nanosilver is considered as the most commonly used engineered nanomaterial in antibacterial textiles,[1] polymeric films for food packaging,[2] paints and pigments,[3] filters for water[4] or air treatment,[5] and so on. It has attractive biomedical applications, taking the advantage of (*a*) antimicrobial properties to prevent infections, and (*b*) plasmonic and metallic properties as a diagnostic (e.g. biosensors, in vivo biomarkers) and therapeutic tool (e.g. photothermal tumor treatment).

Nanosilver can be made by wet- and, in particular, gas-phase routes, which are readily scalable even at academic laboratories.[6]

Reports on epidemic nosocomial infections caused by *Staphylococcus aureus* are frequent, usually originating from hospital environments and cross-contamination that is often associated with the incorrect use of medical equipment,[7,8] which could be minimized by the use of medical devices coated with antimicrobial agents, such as silver nanoparticles (AgNPs).

Studies in the field of medicine have shown that silver is effective on more than 650 pathogens, having a broad spectrum of activity. Its use in the form of nanoparticles enhances this property, granting a wide range of applications.[9,10] Therefore, nanosilver is now considered as one of the most viable alternatives to antibiotics because it seems to have high potential to cope with multidrug resistance, which is often observed in several bacterial strains.[11,12]

The purpose of this study was to evaluate the antimicrobial effects of zinc oxide nanoparticles on human bacteria.

Materials and Methods

Preparation of Zinc Oxide Nanoparticles

Commercially available zinc oxide nanoparticles were purchased from Iranian Nanomaterials Pioneers Company. The size of the nanoparticles was 10-30 nm with a molecular weight of 81/37.

Preparation and Storage of Bacteria

The strains of *Vibrio cholera, Micrococcus luteus, Staphylococcus epidermis, Bacillus cereus* and *Listeria monocytogenes* were supplied from Iranian Fungal and Bacterial Collection. They were incubated for 24 hours at 37°C in nutrient broth medium (liquid medium). After 24 hours, they were stored in a broth culture medium containing 10% sterilized glycerol in a freezer at -20°C for later use.

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Preparation of Bacterial Suspension

Streptococcus pyogenes ATCC® 19615™, *Streptococcus pneumonia* ATCC 49619, *S. saprophyticus* ATCC®15305, Hafnia alvei ATCC 51873, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Serratia marcescens* ATCC 274, and *S. aureus* ATCC® 25923 were cultured in nutrient broth medium after thawing at 37°C in order to verify pure colonies from bacterial samples on a solid TSA medium and incubated for 24 hours at 37°C. After 24 hours, the pure colonies of each bacterium were removed and the opacity was adjusted to MacFarland 0.5 in sterile distilled water. Then, its absorption with a wavelength of 600 nm was read using the UV visible spectrophotometer to ensure the concentration of bacteria. The density of bacteria with a concentration of $1/5*10^6$ CFU/mL had an absorption of 0.08-0.1.

Determining the Sensitivity of Bacterial Strains to Zinc Oxide Nanoparticles

The susceptibility of bacterial isolates to zinc oxide nanoparticles was determined by dilution method in wells. Six wells were prepared in a solid culture medium and 100 µL of each well was added to the Mueller-Hinton Broth (MHB) nutrient medium. Then, 100 mL of a diluted solution of the extracts of plants was added to the first well, and after mixing, 100 mL of the first well was removed and added to the second well, and this was done until the last well. One hundred microliters of the culture medium was removed from the last well and 10 µL of a microbial suspension containing 107 units per mL, equivalent to 0.5 McFarland, was added to it and incubated at 37°C for 24 hours. The first well that prevented the growth of the bacteria after insertion into the incubator was considered as the minimum inhibitory concentration. Ten microliters of transparent wells was removed and transferred to the Mueller-Hinton agar medium, and after 24 hours, the first dilution that could destroy 99.9% of the bacteria was considered as the minimum bactericidal concentration.

Statistical Analysis

One-way ANOVA was used to determine the significant difference between different treatments and then Duncan multiple range test ($P<0.05$) was performed. All the statistical analyses were done using the Statistical Package for Social Sciences (SPSS, version 18.0) for Windows.

Results

The results of this study showed that the maximum inhibitory concentration of zinc oxide nanoparticles was 1500 µg/mL against *S. aureus* and *S. pyogenes*. While the minimum inhibitory concentration was 187 µg/mL against *A. baumannii*. The maximum bactericidal concentration was observed 3000 µg/mL against *S. aureus*. While the minimum bactericidal concentration was observed against *A. baumannii* (Table 1).

Table 1. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of Zinc Oxide Nanoparticles Against Gram-Positive and Gram-Negative Bacteria (µg/mL)

Bacteria	MIC	MBC
Streptococcus pyogenes	1500	1500
Streptococcus pneumoniae	750	1500
S. saprophyticus	375	750
Hafnia alvei	750	1500
Enterococcus faecalis	375	375
Proteus mirabilis	375	750
Serratia marcescens	750	1500
Staphylococcus aureus	1500	3000
Acinetobacter baumannii	187	375

Discussion

Nanosilver has the capacity to be used in biosensing. The plasmonic properties of nanosilver are dictated by its shape, size, and the dielectric medium that surrounds it. Its properties exploited in the dielectric medium make it an ideal candidate for biosensing. Nanosilver biosensors can effectively biosense a large number of proteins, whom normal biosensors find hard to detect. This unique advantage of nanosilver can be utilized in detecting various abnormalities and diseases in the human body including cancer.

The study of Rezaie Keikhaie et al showed that the highest inhibitory concentration against *P. aeruginosa* was 1500 µg/mL, with 4 bacterial strains being inoculated. Moreover, the results showed that the highest drainage concentration was 3000 µg/mL, and 2 strains were inhibited in this concentration and the lowest trap concentration was 93 µg/mL. The lowest inhibitory concentration of plant extract was 0.62 mg/mL, with only 1 strain being inhibited in this concentration. The highest inhibitory concentration against *P. aeruginosa* was 40 mg/mL, with four bacterial strains being inoculated.[13]

In the study of Salomoni et al, the antimicrobial activity of commercial 10-nm AgNPs on two hospital strains of *P. aeruginosa* resistant to a large number of antibiotics and a reference strain from a culture collection was assessed. All strains were susceptible to 5 µg/mL nanoparticles solution. Reference strains INCQS 0230 and P.a.1 were sensitive to AgNPs at concentrations of 1.25 µg/mL and 0.156 µg/mL, respectively; however, this was not observed for hospital strain P.a.2, which was more resistant to all antibiotics and AgNPs tested.[14]

In the study of Kvitek, AgNPs exhibited lower acute ecotoxicity against the eukaryotic organisms such as *Paramecium caudatum*, *Monoraphidium* sp. and *Drosophila melanogaster*. AgNPs were toxic to these organisms at higher concentrations of silver (>30 mg/L).[15]

A lot of reports suggest that the mechanism of toxicity of AgNPs is similar to silver ions, due to the life cycle of AgNPs and their transformation to silver ions.

Conclusion

The antimicrobial activity of silver is well known. In its nanometric form, this characteristic is accentuated. Due to their size, AgNPs can enter cells and inhibit enzymatic systems in the respiratory chain of some bacteria, thereby altering their DNA synthesis. These results support the importance of further studies on using nanosilver to control nosocomial infections caused by strains resistant to most antibiotics. Based on the results presented in this work concerning the action of commercial AgNPs, their use can be recommended as a good alternative for the control of microorganisms, with less risk of toxicity to mammalian cells.

Conflict of Interests

None.

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