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Antibacterial Activity of Silver Nanoparticles

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A B S T R A C T

Bacterial infections are a major cause of chronic infections and mortality. Drug resistant microorganisms pose a serious public health problem and strategies for controlling bacterial activity are needed. Nanoparticles have emerged as a novel alternative to overcome bacterial multidrug resistance due to misuse and abuse of antibiotics. Their use as antimicrobial agents could overcome mechanisms of bacterial resistance as the microbicidal nature of nanoparticles result from direct contact with the bacterial cell wall, inhibiting cellular adhesion and attachment, without the need to penetrate into the cell, interfering in bacterial physiology, quorum sensing, and avoiding biofilm development. The physicochemical properties of nanoparticles are significant elements that regulate their antibacterial actions. Moreover, environmental conditions, the bacterial strain, and the exposure time are other major factors that influence their effects. This review focuses on the antibacterial mechanism and effect of silver nanoparticles, as well as the potential use as an alternative antimicrobial agent.

Introduction

The properties of metal nanoparticles (NPs) have been widely studied for their antimicrobial activity. The most tested metallic NP's are silver, copper, gold, aluminum, titanium, iron, and zinc (Seil *et al.*, 2012; Aderibigbe, 2017).

They have shown broad spectrum antimicrobial activity against Gram positive and negative bacteria, mycobacteria and fungi. However, their antibacterial activity varies among the different types of NPs well as the different microorganisms (Lesniak *et al.*, 2013).

NPs rely on different antibacterial mechanisms when compared to antibiotics; including their size, high surface area, unusual crystal morphologies (edges and corners) and reactive sites (Slavin *et al.*, 2017).

The low surface to volume ratio of NPs can increase their antibacterial activity allowing greater interaction of the nanomaterial with the surrounding environment (Wang *et al.*, 2017).

Antibacterial mechanisms of silver nanoparticles

Amongst the metal NPs, silver nanoparticles (Ag- NPs) have been widely used as an effective antimicrobial agent against bacteria (Alshareef *et al.,* 2017, Adur *et al.,* 2018), fungi (Dojčilović *et al.,* 2017, Kalaivani *et al.,* 2018) and viruses (Etemadzade *et al.,* 2016, Tamilselvan *et al.,* 2017). Silver (Ag) and its compounds have long been used for the disinfection of medical devices and water purification. In medicine, Ag compounds are commonly applied to treat burns, wounds, and a variety of infectious diseases (Avalos *et al.,* 2014).

The use of Ag was nearly abandoned with the discovery penicillin and later on other antibiotics. However, with the emergence of antibiotic-resistant strains, it has regained interest once again (Gaynes *et al.,* 2017). Ag was found to be an efficient bactericidal, antibacterial agent against various pathogens *in vitro* and *in vivo* (de Simone *et al.,* 2014). Moreover, bacteria are less prone to develop resistance against Ag than against conventional antibiotics (Beyth *et al.,* 2015). Classical antimicrobial agents must have the ability to reach vital molecular target sites involved in bacterial metabolism such as cell wall synthesis. In addition, it should avoid ejection by efflux pumps and molecule modification by enzymes. Ag- NPs eliminate bacteria either through their microbicidal effects such as the release of free metal ions, DNA interactions, free radical generation, or by bacterial growth inhibition coupled with the host's immune response. (Hemeg, 2017, Al Matar *et al.,* 2017, Slavin *et al.,* 2017, Bassegoda *et al.,* 2018, Katva *et al.,* 2018)

Cell membrane damage

The contact of Ag-NPs onto the cell membrane of Gram negative bacteria results in the formation of "pits" or "pores" and thus the alteration of the cell membrane permeability, it's rupture and hence depletion of intracellular ATP. (Durán *et al.,* 2016, Akter *et al.,* 2018, Zheng *et al.,* 2018)

Release of toxic ions

Silver ions (Ag+) can react with different groups of proteins in bacteria. Ag -NPs display their antibacterial activity by releasing Ag+ and also by penetrating cells interfering in their metabolic systems. Ag+ ions can also damage DNA by inhibiting its replication. The concentrations required for their bactericidal activity is low (in the range $10^{(-9)}$ mol/l) (Qing *et al.,* 2018).

Interruption of electron transport

The positive charge of Ag + from NPs is critical for its antibacterial activity, as it becomes attached to the negatively charged bacterial cell wall resulting in its rupture, leading to denaturation of proteins and finally cell death. In addition, Ag + can affect membrane-bound respiratory enzymes as well as efflux pumps of ions that also result in cell death. (Dakal *et al.,* 2016, Slavin *et al.,* 2017)

Generation of Reactive Oxygen Species

The oxidizing power of oxygen (O_2) can be lethal for some bacteria. Moreover, the formation of hydrogen peroxide (H_2O_2) by the respiratory burst results in O_2 consumption with the production of free radicals and thus the oxidation of DNA and peroxidation of cellular constituents such as proteins and lipids. Bacteria affected by reactive oxygen species will progressively lose their membrane integrity, rendering them unable to adhere to surfaces, to maintain appropriate communication with other bacteria, nor to efficiently express other cell functions (Durán *et al.,* 2016, Dakal *et al.,* 2016).

Table.1 Factors affecting the antibacterial mechanism of Ag -NPs

No.	Factor	Effect of Ag -NPs	Study
a.	**Particle Size**	Smaller Ag -NPs have larger specific surface areas, which result in a higher antimicrobial activity.	Poulose *et al.*, 2014; Wang *et al.*, 2017
b.	**Particle Shape**	Ag -NPs with different shapes can cause varying degrees of bacterial cell damage.	Van Dong *et al.*, 2012
c.	**Zeta Potential**	Ag -NPs which have a positive surface charge, are prone to being adsorbed on the bacterial surface, in contrast to their negatively charged counterparts.	Halder *et al.*, 2015
d.	**Doping Modification**	Prevent the aggregation of Ag -NPs and allow their dispersal in aqueous environments or other hydrophilic media.	Hartmann *et al.*, 2015
e.	**Roughness**	As the roughness of Ag-NPs increases, the size and the surface area-to-mass ratio promotes the adsorption of bacterial proteins, which is followed by a reduction in bacterial adhesion.	Wang *et al.*, 2017
f.	**Environmental Conditions**	Different environmental conditions cause significant differences in antimicrobial activity such as temperature and pH.	Wang *et al.*, 2017

Table.2 Antibacterial Activity of Ag -NPs against Common Pathogenic Bacteria

Pathogenic Bacteria	Study
Acinetobacter baumannii	Lysakowska *et al.*, 2015; Wan *et al.*, 2016; Singh *et al.*, 2018; Chen *et al.*, 2019;
Escherichia coli	Wang *et al.*, 2014, Dhas *et al.*, 2014; Paredes *et al.*, 2014; Shalaby *et al.*, 2015; Vu *et al.*, 2018
Enterococcus faecalis	Wu D *et al.*, 2014; Alabdulmohsen and Saad, 2017; Chandra *et al.*, 2017; Halkai *et al.*, 2018
Klebsiella pneumonia	Kumar *et al.*, 2016; Chhibber *et al.*, 2017; Hosny *et al.*, 2017; Acharya *et al.*, 2018
Pseudomonas aeruginosa	Das *et al.*, 2016; Yuan *et al.*, 2017; Salomoni *et al.*, 2017;Yan *et al.*, 2018; Punjabi *et al.*, 2018; Liao *et al.*, 2019
Staphylococcus aureus	Paredes *et al.*, 2014; Shalaby *et al.*, 2015; Adibhesami *et al.*, 2017; Kang *et al.*, 2019
Mycobacterium tuberculosis	Sarkar *et al.*, 2015; Punjabi *et al.*, 2018

Factors affecting the antibacterial mechanism of Ag -NPs

Several factors may play a role regarding the antibacterial activity of Ag- NPs against commonly encountered pathogenic bacteria (Table 1) Peptidoglycan plays an important role in hindering the activity of the Ag- NPs, suggesting that a reduction of peptidoglycans in Gram-negative bacteria may increase their

susceptibility when exposed to Ag- NPs.On the other hand, the thick peptidoglycan layer of *Staphylococcus aureus* (*S.aureus)* compared to *Escherichia coli (E. coli)*, prevents the penetration of Ag- NPs into the bacteria. (Wang *et al.,* 2017) In addition the bactericidal activity of Ag-NPs with smaller dimensions (<30 nm) were found to be more effective against *Klebsiella pneumonia (K. pneumoniae) and S.aureus. (*Van Dong *et al.*, 2012; Wang *et al.,* 2017) Hence, the antimicrobial effect of nanoparticles can be related to the interaction with the bacterial surfaces when dealing with Gram-positive strains, whereas for Gram-negative the penetration of the particles can be expected to take place depending on size, charge and other features of the material (Halder *et al.*, 2015; Wang *et al.,* 2017).

Antibacterial effect of Ag -NPs

Several studies have demonstrated the antibacterial activity of Ag-NPs against the most commonly encountered pathogenic bacteria. (Table 2)

Synergistic application of Ag -NPs with antibiotics

It has been reported that as compared to the application of Ag-NPs alone, the combination of antibiotics with Ag-NPs complexes will release Ag + at a higher rate.

Moreover, it has been proposed that the combination of antibiotics with Ag-NPs renders the active groups of antibiotics such as hydroxyl and amine groups to result in the conjugation of both molecules, thus increasing in the effective concentration of antibiotics at a specific site and thus producing a synergistic effect that may overcome antibacterial resistance (Dixit *et al.,* 2017, Kumar *et al.,* 2016, Rajora *et al.,* 2016, Kumar *et al.,* 2018).

Wan *et al., 2016,* reported the synergistic effects of combining Ag-NPs and Polymyxin B or Rifampicin against drug-resistant *Acinetobacter baumannii* isolated from clinical patients. Kanamycin and Rifampycin were combined with Ag-NPs at a low concentration (5 µg/disk), and were tested against five foodborne pathogens; namely *Bacillus cereus, Listeria monocytogenes, S.aureus, E.coli* and *Salmonella typhimurium*. When both antibiotics and Ag-NPs were mixed, they displayed strong antibacterial activity against all pathogens, with zones of inhibition ranging in diameter from 10.62 to 14.33 mm in contrary to no inhibitory effect when applied alone at the same concentration (Patra *et al.,* 2017).

In another study, Gentamicin and Chloramphenicol were combined alone and together with Ag-NPs and were tested against *Enterococcus faecalis*, where the antimicrobial effect of Ag-NPs increased in proportion to the increase in it's concentration (Katva *et al.,* 2018). The synergistic effects of with Amakacin and Ampicillin with enhanced anti-biofilm activity was recorded against multidrug resistant *Pseudomonas aeruginosa, E. coli, K. pneumoniae* isolated from burn wounds in a study conducted by Nasser, 2018.

Although numerous studies reported the effective antimicrobial activity of Ag-NPs alone and in combination with different antibiotics, further studies are required to determine the minimal inhibitory concentration (MIC) of Ag, the emergence of resistant strains, the lethal effect on biofilm, and its side effects on humans. (Baptista *et al.,* 2018)

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