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Review Article

Silver Nanoparticle and their Antimicrobial Properties

Khaled Al-hosaini[1] and Asim Azhar[2]*

[1]*Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia*

[2]*Aligarh College of Education, Aligarh, Uttar Pradesh, India*

***Corresponding Author:** Asim Azhar, Assistant Professor, Aligarh College of Education, Aligarh, Uttar Pradesh, India.

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Abstract

Silver nanoparticles possess broad spectrum antimicrobial properties with evident applicability in biomedical and industrial endeavors. They can be fabricated and modified appropriately in the desirable manner to utilize at their maximum potential. The most noticeable nanoparticle for medical intervention are silver nanoparticles which are renowned for their high antimicrobial activity. Silver ion has been acknowledged as a metal ion that demonstrate anti-mold and anti-algal properties for long time. In the present review, we report antimicrobial properties of silver nanoparticles characterized by several biophysical techniques and their mechanism through generation of reactive oxygen species (ROS).

Keywords: *Antibacterial; Nanoparticle; Nanotechnology; Reactive Oxygen Species; Silver*

Abbreviations

NPs: Nanoparticles, H_2O_2: Hydrogen Peroxide

Introduction

Nanotechnology, an emerging and pioneering multidisciplinary technology with physics, chemistry, biology, material science and medicine [1]. The prefix 'nano' is derived from Greek word 'nanos' meaning "dwarf" that denotes to things of one-billionth of meter (10^{-9}m) in size. The main conception of nanotechnology was presented by 'Richard Feynman' in a talk entitled as "There's plenty of room at the bottom" at the American physical society meeting at Caltech in 1959. Nanotechnology has been incorporated in various industrial segments because of its presentations in the field of biotechnology [2], targeted drug delivery [3], vehicle for gene and drug delivery and industrial applications. Therefore, with wide range of applications accessible, these particles have tremendous potential to make a substantial impact on human life.

Silver has been valued as an antimicrobial since Egyptian times. In the present time, silver compounds are extensively used as an antimicrobial agents particularly in the treatment of wounds and burn injuries [4]. Silver ion (Ag^+) is highly toxic and defined as 'oligodynamic' against a broad spectrum of microorganisms, possibly due to its inhibition of certain oxidative enzymes, protein denaturation, or interfering with DNA replication [5]. However, silver exhibit low toxicity to human tissues and had provoked only rare instances of bacterial silver resistance while traditional antibiotics has innumerable side effects [6]. The molecular mechanism for antimicrobial activity has been conjectured to stem from the oxidation of metallic silver (Ag^0) to silver cation (Ag^+) on exposure to aqueous solutions possessing oxidizing agents [7]. Silver NPs can be oxidized to Ag^+ but cannot be reduced due to their outermost electronic configuration ($4d^{10}$, $5s^1$).

The surface of silver nanoparticle is unprotected to aqueous oxidants giving rise to Ag^+, thus deactivating proteins necessary for bacteria, viruses and fungi to survive. The controlled release of Ag^+ ions from silver nanoparticle avoids the excess delivery of Ag^+ ions. The cytotoxicity of Ag NPs against broad range of microorganisms varies with the quantum of silver ions released into the surrounding [8]. In the current review, we have summarized few important antimicrobial functions of the silver nanoparticles. We then discuss the general methods of synthesis of NPs from bacteria, fungi and plants. We also present the general mechanism of inhibition of silver nanoparticles against the bacterial cell.

Synthesis of different types of silver nanoparticles

Principally, metal nanoparticles are prepared from two methods, first is 'bottom-up' and second one is 'top-down'. In bottom-up method, building of nanoparticle is considered from the bottom, i.e. atom by atom, molecule by molecule and cluster by cluster while in the top-down approach, successive cutting of a bulk material is performed to form nano-sized particles [9]. The bottom-up method is preferred for nanoparticle synthesis due to their involvement of homogeneous system where catalysts (for example, enzymes and reducing agent) are controlled during the process. Numerous procedures are offered for the synthesis of silver nanoparticles; for example, physical, chemical and biological methods [10]. Further, chemical method of synthesis can be subdivided into electrochemical, chemical reduction, pyrolysis and irradiation-assisted chemical methods [11]. In solution, silver nanoparticle synthesis requires metal precursors, reducing agents, stabilizing and capping agent. Frequently used reducing agents are ascorbic acid, borohydride, alcohol, sodium citrate and hydrazine. In physical methods, harmful chemicals are not utilized in the process and processing time is fast as compared to chemical methods. Another advantage of physical methods over chemical methods is the narrow size distribution of nanoparticles [12]. The main drawback of physical method is the consumption of high energy in the preparation of nanoparticles. To overcome the drawbacks like high energy consumption and harmful handling of chemicals, biological synthesis of silver nanoparticle from herbal extract and microorganisms have been preferred as an alternative approach due to their plethora of advantages. It is now well established that biological method is simple, cost-effective, eco-friendly and easily scalable for high yield or production [13]. In the last decade, biosynthesis of metal nanoparticles utilizing biological organisms like bacteria, fungi, yeast and algal extracts have expanded recognition in the field of nanotechnology.

The parts of the plant usually possess carbohydrates, fats, proteins, nucleic acids, pigments as well as secondary metabolites which act as reducing agents to synthesize NPs from metal-ions without yielding any lethal by-products. The biomolecules (enzymes, proteins and bio-surfactants) present in the microorganisms assist as a reducing agents.

Extracellular production of silver nanoparticles encompasses, the entrapping of metal ions on the exterior surface of the cells and reducing them in the presence of enzymes or biomolecules, whereas intracellular synthesis occurs inside the microbial cells. Recent research have presented that the extracellular synthesis of NPs is inexpensive, helps large scale production and needs simpler downstream processing; thus extracellular method is preferrable over intracellular [14].

The potential of biosynthesis of silver nanoparticles employing bacteria have been comprehended in recent years [15]. For example, *Pseudomonas stutzeri* (AG259) isolated from silver mine was frequently used to manufacture silver nanoparticles within the cells [16]. Furthermore, numerous bacterial strains like *A. calcoaceticus*, *B. amyloliquefaciens*, *B. flexus*, *B. megaterium* and *S. aureus* (Gram -ve and Gram +ve) have been employed for the biosynthesis of silver nanoparticle (intracellular as well as extracellular) [17]. The biophysical characteristics of these synthesized NPs from culture supernatant, aqueous cell free extracts or cells are spherical, disk, cuboidal, hexagonal as well as triangular in shape. In 2009, Saifuddin., *et al*. exhibited biosynthesis (extracellular) of silver NPs possessing size in the range 5 - 50 nm exploiting a mixture of culture supernatant of *B. subtilis* in microwave irradiated water. The culture supernatants of *K. pneumonia*, *E. coli*, and *Enterobacter cloacae* have also been used for the rapid biosynthesis (time span ~ 5 min) of silver nanoparticle as recently reviewed by Jannathul., *et al*. [18]. Table, we presented various types of bacteria-mediated synthesis of silver nanoparticles and their mechanism of action.

S. No	Name of Bacteria	Mechanism of Action	Size and Shape	Location	References
1.	*E. Coli*	Affects permeability of the membrane and respiration	42 - 89 nm, spherical	Extracellular	[19]
2.	*Acinetobacter haemolyticus* (MMC8)	Interferes with growth	4 - 40 nm	Extracellular	[20]
3.	*Acinetobacter baumannii*	Affects cell wall and cytoplasm	133 nm	Intracellular	[21]
4.	*Acinetobacter calcoaceticus*	NA	8-12 nm, spherical	Extracellular	[22]
5.	*Klebsiella pneumoniae*	Modification of membrane	15- 37 nm, spherical	Extracellular	[23]
6.	*Staphylococcus aureus*	Permanent damage on bacterial cell	60 nm, nanosphere	Extracellular	[24]
7.	*Listeria monocytogenes*	Morphological variations, splitting of the cytoplasmic membrane from the cell wall, plasmolysis	23 nm, spherical and polygonal	Extracellular	[25]
8.	*Enterococcus faecalis*	Modification of cell wall and cytoplasm	10 - 80 nm	Extracellular	[26]
9.	*Micrococcus luteus*	Affects membrane	5 - 15 nm	Extracellular	[27]
10.	*Enterobacter aerogenes*	Interferes efflux pump	25 - 35 nm, spherical	Extracellular	[28]
11.	*Gluconobacter roseus*	NA	10 nm	Extracellular	[29]
12.	*Morganella* sp.	NA	10 - 40 nm, quasispherical	Extracellular	[30]
13.	*Pseudomonas aeruginosa* (SM1)	Disrupt the cell membrane	6.3-4.9 nm, spherical disk shaped	Extracellular	[31]
14.	*Rhodobacter spheroides*	NA	3-15 nm, spherical	Extracellular	[32]
15.	*Vibrio alginolyticus*	NA	50-100 nm, spherical	Extracellular	[33]
16.	*Xanthomonas oryzae*	NA	14.86 nm, spherical, triangular	Extracellular	[34]
17.	*Proteus mirabilis*	Free radical generation	10-20 nm, spherical	Extracellular and Intracellular	[35]
18.	*Salmonella typhi*	Interferes with bacterial DNA replication, damages cytoplasmic membrane, changes in intracellular ATP levels	1-10 nm, icosahedral and decahedral	Extracellular and Intracellular	[36]

Table 1: *Bacteria-mediated synthesis of silver nanoparticles and their mechanism of action.*
NA: Not applicable.

Both pathogenic as well as non-pathogenic fungi has been widely explored for the biosynthesis of Ag NPs [37]. In the presence of fungi, silver ions are reduced extracellularly to generate stable silver NPs in water [38]. Thermophilic fungus (*Humicola* sp.) have also been exploited for extracellular synthesis of silver NPs previously reported by Syed., *et al*. [39]. Tree oyster mushroom (*Pleurotus ostreatus*) have been recently described for biogenic synthesis of silver NPs [40]. Numerous fungi like *Aspergillus flavus*, *A. fumigatus*, *Fusarium oxyporum*, *F. acuminatum*, *F. culmorum*, *F. solani*, *Phoma glomerate*, *Phytophthora infestans*, *Metarhizium anisopliae*, *Trichoderma viride* and *Vertcillium* sp. have been regularly exploited for both extra - as well as intra - cellular biosynthesis of silver nanoparticles [41]. The synthesized NPs are found in various shapes and sizes.

Cytotoxicity of silver nanoparticles

The size, shape, capping agent and the type of pathogen against which their toxicity is tested, determines the cytotoxicity of the nanoparticles. The toxicity of NPs synthesized from green methods as well as non-green methods has been evaluated extensively. It was found that NPs synthesized from green methods are more toxic as compared to non-green methodologies. NPs slowly encircle the microbes and enter into the cell, preventing their essential functions. Nanoparticles are comparatively more toxic than bulk material as they act at cellular, subcellular and biomolecular levels [42]. It has been believed that the cytotoxicity of silver NPs is due to the production of ROS; and as a result, reduction of glutathione level has been observed. The effect of silver NPs on cultured cells *in vitro* was reported by Kim and Ryu., *et al*. where an increase in oxidative stress, apoptosis and genotoxicity was noticed [43].

Molecular mechanism of silver nanoparticles

The precise mechanism through which silver nanoparticles work to produce antimicrobial outcomes is still the area of further intense research. However, several concepts on the action of silver nanoparticles on microorganisms to cause the microbicidal effect are considered. Silver nanoparticles possess the capability to anchor the bacterial cell wall and afterwards penetrate it, thus causing structural changes in the cell membrane; thus increasing the permeability of the cell membrane, ultimately leading to the death of the cell. The formation of 'pits' on cell surface occurs besides accumulation of the nanoparticles on the periphery of the cells [44]. In figure 1, we delineate the ROS mediated mechanism of silver NPs on bacterial cell. NPs usually trigger toxicity by the formation of free radicals such as ROS [45]. The ROS comprises of superoxide (O_2^-), hydroxyl (^-OH), peroxy ($RCOO^-$) and hydrogen peroxide (H_2O_2). The resulting ROS may cause membrane disruption and disturb the permeability as well as integrity of the membrane. Various processes of NPs like electrostatic interaction, adsorption and penetration come into play in the growth inhibition mechanism of bacterial cell wall. The replication of is severely affected, microbe limiting its ability to survive in ROS rich environment will no longer be able to survive. However, in the presence of antioxidant (enzyme), which scavenge the free radicals may delay the process of growth inhibition [46]. The composition of NPs, surface modification, intrinsic properties and type of microorganism determines the toxicity [47]. Recently. Yan Xu., *et al*. demonstrated the antibacterial properties of Ag NPs in model organism *P. aeruginosa* (Gram negative bacteria) and found that there are more than fifty silver regulated proteins as well as five silver binding proteins [48]. *In silico* analysis revealed the production of intracellular ROS and its interference with the cell membrane function. It has been well documented that silver NPs carry out their anti-bacterial outcome through the modulation of signal transduction pathways [49].

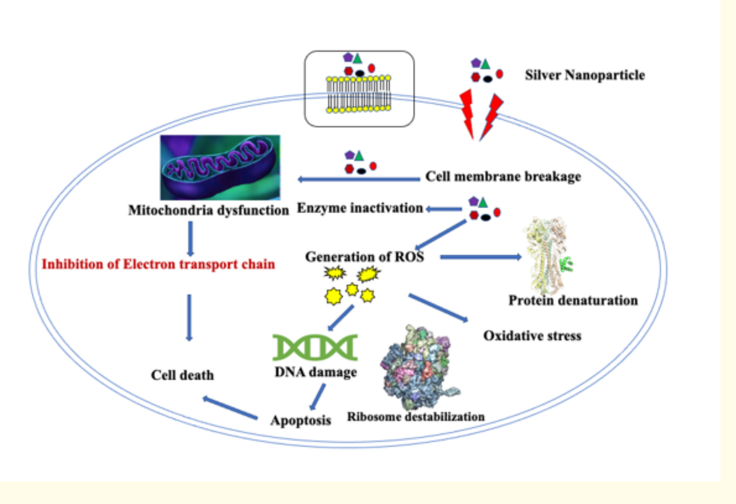

Figure 1: *Reactive oxygen mediated antimicrobial action of silver nanoparticles leading to cell death [49-50].*

Reason behind consideration of silver for various purposes

Traditionally, metallic silver has been utilized through ages for different reasons. Many societies use silver as ornaments, jewelry and fine cutlery in daily life. In ancient Ayurveda (traditional Indian medical system), silver has been utilized as beneficial agent for numerous sicknesses. There is an increasing demand for silver as an effective antibacterial, antifungal agent in burn/wound care system. In medical and dental surgical items, silver has been widely considered for coating the devices to keep away the infections at bay. For the treatment of mental illness (epilepsy), nicotine addiction, stomatitis, gastroenteritis and sexually transmitted diseases like syphilis and gonorrhea, soluble silver compounds have been exploited.

Conclusion

Biomedical application of silver nanoparticle has gained tremendous space in the scientific research in last two decades. Nanoparticles possess the essential capability to cross the membrane and disrupt the major functions of the microorganisms through ROS medicated activation mechanism. Nanoparticles are also exploited to load the drug of the interest to cross the membrane and deliver the cargo within the cell. Toxicity of nanoparticles are one of the major issue that needs to be addressed before effective and safe delivery of NPs. Further, *in vitro* as well as *in vivo* research needed to perform before any clinical application of the nanoparticles.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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