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Recent Advances in the Delivery, Mechanism of Action and Antibacterial Activity of Silver Nanoparticles

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Abstract

Nanoparticles, especially silver nanoparticles (AgNPs), have revolutionized various fields like microbiology, biotechnology, pharmacy, and medicine owing to their distinct properties. This research delves into the significant potential of AgNPs in antimicrobial therapy, focusing on recent advancements in their delivery mechanisms, mechanisms of action, and antibacterial efficacy. The effective targeted delivery of AgNPs to specific body sites remains a challenge, leading to innovative approaches in nanotechnology. Nanogels, liposomes, and polymer-based nanoparticles have emerged as promising delivery systems, enhancing the stability, bioavailability, and controlled release of AgNPs. The antimicrobial activity of AgNPs is rooted in their unique physicochemical properties, such as high surface area and reactivity. They disrupt bacterial cell membranes, increasing permeability, causing cell death, and interfering with intracellular components. Additionally, AgNPs have shown potential in inhibiting biofilm formation, a common defense mechanism of bacteria against antibiotics. Despite their promise, addressing issues related to cytotoxicity and refining delivery methods remains imperative. This review comprehensively addresses the challenges associated with the delivery of AgNPs, their cytotoxic effects, and their efficacy against antibiotic-resistant bacteria, highlighting their mechanism of action in bacterial eradication and biofilm inhibition.

Keywords: Anti-microbial resistance, Delivery, Mechanism of action, silver nanoparticles

INTRODUCTION

A thorough literature search focusing on the delivery, mechanism of action, and antibacterial activity of silver nanoparticles published within the last 5-10 years was carried out. Online databases such as PubMed, Scopus, Web of Science, and Google Scholar were utilized. Keywords such as "silver nanoparticles", "delivery", "mechanism of action", "antibacterial activity", "nanotechnology", and "nanomedicine" were used to refine the search results and select pertinent articles for inclusion in the review.

ANTIBACTERIAL MECHANISM OF SILVER NANOPARTICLES

Much research has shown that AgNPs possess antimicrobial capabilities against a range of resistant bacteria. These include *Klebsiella pneumoniae* (Fontoura *et al.*, 2023), hypervirulent *Klebsiella* pneumoniae (Lan *et al.*, 2022), *Streptococcus pyogenes* (Avire *et al.*, 2021), *ampicillin-resistant E. coli* (Ehsan *et al.*, 2023), *multidrug-resistant P. aeruginosa*, *Listeria monocytogenes*, Methicillin-resistant *Staphylococcus aureus*, erythromycin-resistant, and *K. aerogenes* (Dove *et al.*,2023). The diversity of antibacterial activity of AgNPs cuts across many bacteria, and the action can be bactericidal and/or bacteriostatic (Ahmed et al., 2023; Ernest et al., 2014a). The mechanisms employed by AgNPs to induce bactericidal and bacteriostatic action against bacteria include but are not limited to the following:

Generation of Reactive Oxygen species (ROS)

The process by which AgNPs induce the generation of ROS in bacteria starts with penetration and release of silver ions. These silver ions are chemically reactive and can interact with various cellular components (Li *et*

al.,2021). One of the primary mechanisms for ROS generation involves the impairment of the bacterial electron transport chain (ETC) (Li *et al.*,2020). This interruption can occur through several processes such as cell membrane damage, DNA damage, lipid peroxidation, deactivation of superoxide dismutase, induction of reactive stress, and generation of reactive oxygen species. Silver ions can interact with proteins and enzymes involved in the electron transport chain. This interaction can disrupt the flow of electrons along the chain (Faúndez *et al.*,2023). The disruption of the ETC can lead to the leakage of electrons from the chain. These leaked electrons can then react with oxygen (O_2) to form superoxide radicals. Silver ions can also directly interact with oxygen molecules to form superoxide radicals and other ROS (Dryden, 2018). As highlighted in the equation below:

$$Ag^{+} + O_2 \rightarrow Ag + O^{2\bullet-}$$

The combined effects of disrupted ETC components and direct interaction with oxygen result in the accumulation of ROS within the bacterial cytoplasm. The increased levels of ROS overwhelm the bacterial natural antioxidant defense systems, which include enzymes like superoxide dismutase and catalase. This imbalance results in oxidative stress. ROS are highly volatile and can impair various cellular components, including lipids, proteins, and DNA, leading to protein oxidation, lipid peroxidation, and DNA strand breaks. The accumulation of oxidative damage disrupts essential cellular functions and processes, ultimately causing cell dysfunction and death (Nallanthighal *et al.*,2017).

Technically, ROS generation by silver nanoparticles leads to almost all antibacterial activity secondary to ROS production, which can result in cell membrane damage, cytoplasm leakage, DNA damage, oxidative stress, lipid peroxidation, and deactivation of ATP production (Mikhailova, 2020).

Oxidative Stress

Silver nanoparticles trigger harmful effects by producing ROS and initiating oxidative stress. Various mechanisms have been suggested to explain how metal nanoparticles exert their antibacterial effects. Among these, two frequently mentioned mechanisms involve the internal toxicity caused by released metal ions and the promotion of oxidative stress through elevated ROS. Another mechanism of oxidative stress induced by AgNPs in bacteria involves the generation of ROS and disruption of the cellular antioxidant defense systems (Mammari *et al.*,2022). Membrane damage and cytoplasmic leakage, secondary to the production of ROS, disrupt antioxidant activity by interfering with the superoxide dismutase (SOD) enzyme, thereby inducing oxidative stress and damaging all cell organelles within the cytoplasm. Ultimately, the buildup of ROS results in oxidative stress, which ultimately leads to the demise of the cell (Ameh *et al.*,2022).

Triclosan-Like Antibacterial lipid peroxidation

Triclosan functions by inhibiting lipid biosynthesis through its binding to the enoylacyl carrier protein reductase enzyme (ENR). This binding effectively hampers the microbe's ability to produce the necessary fatty acids for lipid production (Bibens *et al.*,2023). RNA sequencing data revealed that silver nanoparticles (AgNPs) triggered a bactericidal mechanism similar to triclosan, obstructing type II fatty acid biosynthesis. Additionally, the silver ion induced oxidative stress both inside and outside of K. pneumoniae cells (Pareek *et al.*,2021). Binding to ENR also leads to direct lipid peroxidation by initiating ferroptosis, which targets oxidant lipids. Uncontrolled lipid peroxidation results in the generation of hydroperoxides, lipid peroxyl radicals, and various oxidation byproducts, ultimately causing membrane rupture and cell death (Endale *et al.*,2023).

Cell membrane Damage

Silver ions (Ag+) released adhere to the bacterial cell wall and cytoplasmic membrane (More *et al.*,2023a). Multiple studies propose that they mainly target the cell membrane (Yin *et al.*,2020). In gram-negative bacteria, AgNPs primarily damage the cell wall at the plasma membrane, while the outer membrane is not impacted by the nanoparticles. The accumulation of silver nanoparticles denatures the cell membrane (More *et al.*,2023b). Additionally, due to their size, AgNPs can penetrate the cell walls, altering the structure of the cell membrane (Fernandes *et al.*,2023). This membrane denaturation can result in the disruption of subcellular organelles and even lead to cell destruction. Moreover, AgNPs can influence bacterial signal transduction that relies on phosphate transfer to protein substrates, and nanoparticles can remove phosphate from tyrosine residues on these peptide substrates. This disruption of signal transduction can trigger cell apoptosis and halt the multiplication of cells (Yin *et al.*,2020).

This mechanism that led to the destruction of bacterial membranes was observed in Gram-negative bacteria, where membrane proteins facilitate the uptake of silver ions into the cell. For instance, intracellular synthesis of AgNPs has been confirmed in *Enterobacter* cloacae (Mikhailova, 2020). Similar findings have been reported for *Pseudomonas stutzeri* (Rajora *et al.*,2016). This mechanism was also detected in *Corynebacterium sp*ecies and various other bacteria belonging to the Streptomyces genus. Among fungal representatives, *Verticillium sp*ecies are notable for their ability to synthesize AgNPs. Additionally, certain microorganisms can carry out AgNP biosynthesis both inside and outside the cell, including *Bacillus strain CS 11 and Proteus mirabilis* (Mikhailova, 2020).

Cytoplasmic leakage

The leakage of bacterial cytoplasmic contents may result from both extracellular and intracellular effects, primarily damage to the cellular membrane by nanoparticles (Yao *et al.*,2023). Several studies have demonstrated that nanoparticles can disrupt the structural integrity of microbes by interacting with outer cell wall components, leading to cell leakage and eventual cell death. Nanoparticles have the capacity to interfere with protein structures, causing damage to the cellular membrane and resulting in cell leakage and death. The presence of nanoparticles can induce ROS generation at the bacterial cell interface, further stressing the cell membrane, causing cytoplasmic leakage, and ultimately leading to bacterial cell death (Lohans *et al.*,2019). Sahoo (Sahoo *et al.*,2023) reported that qualitative and quantitative measurements of bacterial cytoplasmic content indicated its induction by nanoparticles. Furthermore, advanced microscopic analyses conducted by Modi (Modi *et al.*,2023) demonstrated increased polarization, as well as changes in protein and membrane fluidity leading to leakage.

The ability to induce biofilm formation, hinder growth processes, cause intracellular material release, increase membrane permeability, and stimulate the generation of reactive oxygen species may be associated with its smaller dimensions (5.7 nm), uniformity, zeta potential, and electrical conductivity (Kora & Sashidhar, 2015). This is most effective on gram-negative bacteria, specifically active against *Klebsiella* pneumoniae in antibiofilm formation (Scandorieiro *et al.*,2023).

Protein Denaturation

Nanoparticles can denature bacterial proteins through various mechanisms, often involving direct interactions between the nanoparticles and the proteins (Ozdal & Gurkok, 2022). Silver nanoparticles can interact with the surface of bacterial proteins, disrupting their native conformation. This interaction can lead to changes in the protein's secondary, tertiary, and quaternary structures, ultimately rendering the protein nonfunctional (More *et al.*,2023b). Electrical conductivity or electrostatic interactions of silver nanoparticles with bacterial cells may attract and bind to oppositely charged regions on bacterial proteins, distorting the protein's structure and destabilizing its functional state (Ozdal & Gurkok, 2022). Additionally, silver nanoparticles with hydrophobic surfaces can bind to hydrophobic regions on bacterial proteins, leading to protein unfolding and exposure of the protein's hydrophobic core, disrupting its natural structure and causing denaturation (More *et al.*,2023b). Bacterial proteins often contain disulfide bonds that contribute to their stability. Nanoparticles can interfere with these bonds, leading to the breakage of disulfide linkages and subsequent protein denaturation (Habibi *et al.*,2022). Furthermore, silver nanoparticles can physically interact with proteins, exerting mechanical forces that result in protein unfolding and denaturation (Park *et al.*,2023). This unfolding can occur through shear forces, compression, or stretching induced by the physical interactions of silver nanoparticles synthesized from *Cissus* quadrangularis extract with egg albumin (Kanimozhi *et al.*,2022). Most notably, silver nanoparticles and some other nanoparticles can generate ROS when interacting with bacterial cells. ROS can indirectly contribute to protein denaturation by causing oxidative stress and damaging the protein's structure (Endale *et al.*,2023; Li *et al.*,2021).

DNA Damage

Virtually all interactions between silver nanoparticles and bacteria affect multiple organelles within the bacteria, primarily triggered by the generation of reactive oxygen species (ROS) (Faúndez *et al.*,2023). AgNPs can induce the production of reactive oxygen species like O_2 and H2O2, which are highly reactive and capable of damaging cellular components,

including DNA. ROS can result in the formation of oxidative DNA lesions such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Dryden, 2018). The direct binding of AgNPs to DNA molecules can cause physical disruption or destabilization of the DNA double helix, interfering with repair processes, DNA replication, and transcription (More *et al.*,2023b). Subsequent ROS generation due to AgNPs activity can lead to oxidative stress, physical interactions with DNA, or interference with DNA repair mechanisms, resulting in the breakage of both single and double-stranded DNA (Shukla *et al.*,2021). DNA repair pathways like base excision repair (BER) and homologous recombination (HR) are crucial for maintaining genomic integrity. Disruption of these pathways can lead to the accumulation of DNA damage (Nallanthighal *et al.*,2017). According to Chen *et al.*,(2022), AgNPs might induce epigenetic modifications that can alter gene expression patterns. Changes in gene expression can indirectly impact DNA integrity and repair mechanisms, highlighting the diverse factors that can influence how AgNPs damage bacterial DNA.

Alteration of Cell Division

Silver nanoparticles (AgNPs) can disrupt the normal process of cell division through various mechanisms, including mitotic arrest (Kah *et al.*,2023; Takáč *et al.*,2023), chromosomal aberrations (El-Ansary *et al.*,2023), spindle fiber dysfunction (Daphedar & Taranath, 2018), cytokinesis inhibition (Mikhailova, 2020; Takáč *et al.*,2023), DNA damage (Nallanthighal *et al.*,2017), and reactive oxygen species production (Frei *et al.*,2023). These disruptions can have significant implications for cellular health and overall organism well-being. The progression of the cell cycle is a crucial mechanism ensuring DNA replication, cell growth, division, and organism rejuvenation. However, overexpression of growth factors, gene mutations, or impaired regulation of tumor suppressor proteins can lead to cells losing the ability to exit the cell cycle, resulting in uncontrolled cell division (Takáč *et al.*,2023).

Deactivation of ATP synthesis

When bacteria are exposed to different levels of Silver nanoparticle treatment, the expression levels of key genes associated with responses that facilitate ATP synthesis are increased (Xu *et al.*,2023). Silver nanoparticles deactivate ATP synthesis by disrupting several metabolic pathways and the electron transport chain (ETC) responsible for ATP synthesis (Mikhailova, 2020). Silver nanoparticles are typically small enough to breach the bacterial cell membrane. Once inside the bacterial cell, can release silver ions (Ag^+). These ions are chemically reactive and can readily interact with cellular components (Anees Ahmad *et al.*,2020). The bacterial ETC is responsible for generating a proton gradient across the inner membrane, which is essential for ATP synthesis. Silver ions disrupt the ETC by binding to key proteins involved in electron transfer. This binding interferes with the flow of electrons along the ETC (More *et al.*,2023b). In normal bacterial respiration, the ETC pumps protons (H^+) across the inner membrane to create a proton gradient (Yin *et al.*,2020). This gradient drives the ATP synthase complex, allowing it to produce ATP. Silver ions can inhibit the proton-pumping function of the ETC, preventing the establishment of the proton gradient. Silver ions can also directly interact with the ATP synthase enzyme, which is responsible for synthesizing ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi). Inhibition of ATP synthase by silver ions further reduces ATP production. As a result of these multiple interferences, the bacterial cell experiences a significant reduction in ATP production Since ATP is the primary energy currency of the cell, its depletion severely affects the cell's ability to carry out essential metabolic processes and eventually leads to bacterial deactivation (McNeilly *et al.*,2021).

The stepwise antibacterial mechanism responsible for generating ROS is presented in Figure 1 below

Spectrum of bacteria susceptible to silver nanoparticles

Several research studies have confirmed the antimicrobial effectiveness of AgNPs against a range of resistant bacteria. These bacteria include *Streptococcus pyogenes* resistant to erythromycin, *E. coli* resistant to ampicillin, multidrug-resistant *Pseudomonas aeruginosa*, *Listeria monocytogenes, Klebsiella pneumoniae*, and *K.* aerogenes (Dove *et al.*,2023). Gram-negative bacteria tend to be more vulnerable to the action of silver nanoparticles because their cell walls are thinner compared to those of gram-positive strains (Yin *et al.*,2020). These nanoparticles demonstrated their effectiveness against other bacterial pathogens such as *Hemophilus influenzae* and *Streptococcus pneumoniae* as evidenced by their minimum inhibitory concentrations (MICs): 109.4, 120.60, and 138.80 µg/ml for AgNPs, and 105.80, 114.40, and 129.06 µg/ml for AgNPs, respectively.

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Furthermore, these nanoparticles inhibited biofilm formation in *S. pneumoniae, H. influenzae, and N. meningitidis* by 73.14%, 71.89%, and 64.81%, respectively. These results validate the potential of synthesized AgNPs in combating multidrug-resistant (MDR) microbes that cause meningitis and form biofilms (Dove *et al.*,2023). The measured minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the surface-stabilized silver nanoparticles indicate their efficacy as potent antibacterial agents even when used in small quantities.

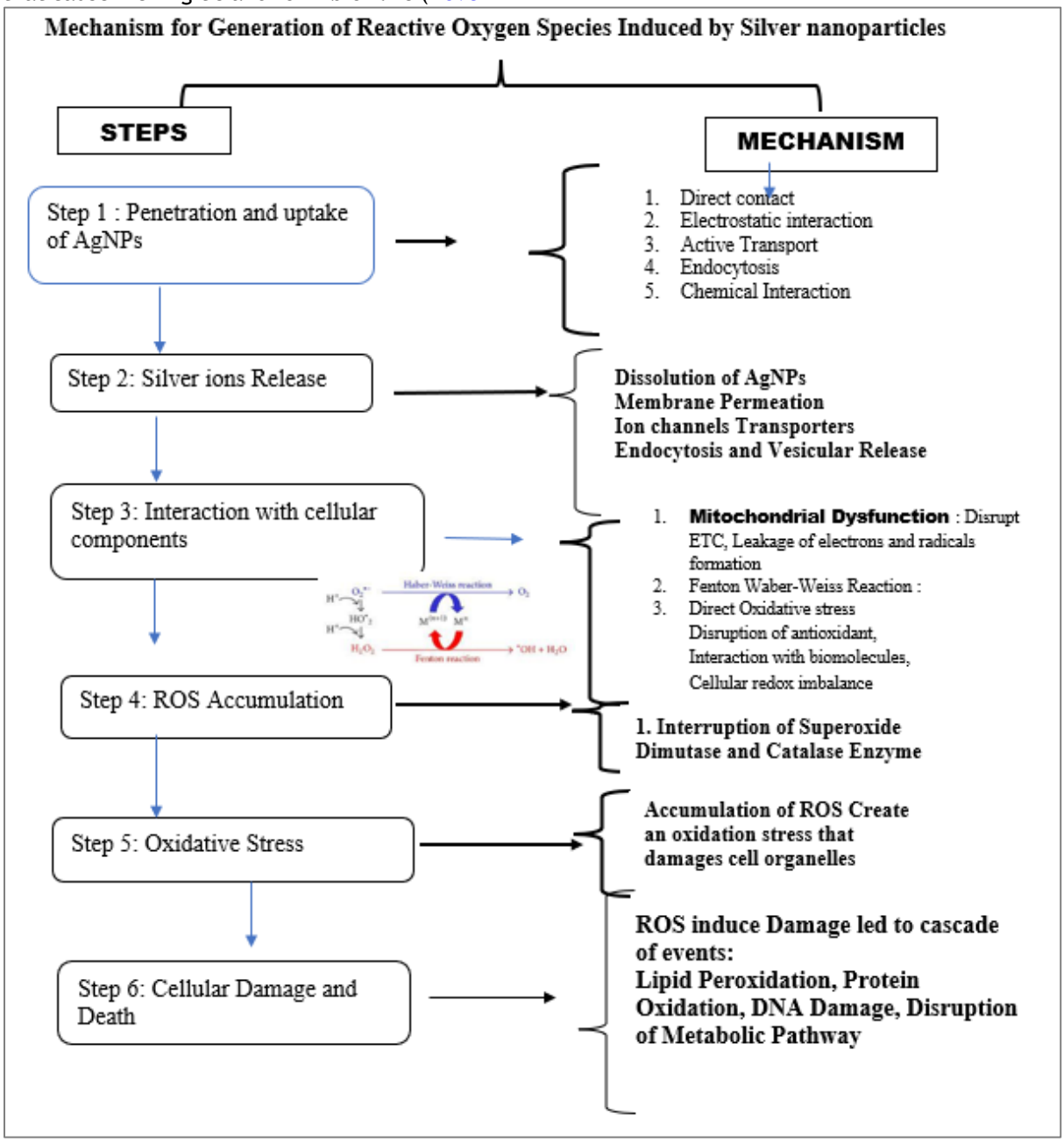

Figure 1: Stepwise Mechanism for the Generation of Reactive Oxygen Species by Silver Nanoparticles (Ameh et al., 2022; Frei et al., 2023; Guo et al., 2016; N. Li et al., 2023; Nakamura et al., 2016)

ANTIMICROBIAL PROPERTIES OF SILVER NANO PARTICLES

Silver nanoparticles exhibit antimicrobial properties, and their potential toxicity can be evaluated by determining several parameters, including Minimum Inhibitory Concentration (MIC) (Bano *et al.*,2023), Minimum Bactericidal Concentration (MBC) (Kim *et al.*,2023), Host Cell

Inhibitory Concentration (IL50), Host Cell Lethal Concentration (LD50) (Dutt *et al.*,2023), and Minimum Cytotoxic Concentration (MCC) (Noga *et al.*,2023). However, it is crucial to acknowledge that various factors can significantly impact MIC, MBC, IC_{50}, LD_{50}, and MCC values. These factors include nanoparticle size, the presence of stabilizing agents, conjugation with other substances, electrical conductivity (EC), pH levels, Total Dissolved Solids (TDS), and the concentration of dissolved oxygen (DO) in the solution where the nanoparticles are present. Recent advancements in determining the MIC and MBC of these nanoparticles are detailed in Table 1 below.

MIC and MBC of Silver Nanoparticles

The minimum inhibitory concentration (MIC) for AgNPs was shown to be effective against both gram-positive and gram-negative nosocomial pathogens using various methods, with the U-bottom, 96-well microtiter plate incubation method being particularly notable. *Enterococcus faecalis* demonstrated inhibition at concentrations ranging from 1.05 µg/mL to 2.5 µg/mL, as reported by Dutt *et al.*,(2023), with a MBC of 10 µg/mL according to Tverezovska *et al.*,2022). *P. aeruginosa* and *S. aureus* were inhibited at concentrations of 1 µg/mL and 2 µg/mL, respectively, as documented by Liao *et* al., in 2019. Conversely, *E. coli, S. aureus,* and *S. multivorum* exhibited inhibition at 0.003 µM, 0.003 µM, and 0.25 µM, respectively, and were killed at 0.031 µM, 0.015 µM, and 0.25 µM when AgNPs were stabilized with cetyltrimethylammonium bromide (CTAB), per Ameh *et al.*,(2023). Dove *et al.*,(2023) reported an *E. coli* with an MBC of 6.2 µg/mL, while the MIC was 3.1 µg/mL. MICs of 1 µg/mL against *P. aeruginosa* and 2 µg/mL against *S.* aureus were also observed for AgNPs. The antibacterial effect of AgNPs was found to be dosage-dependent based on the results of the cell viability assay, with MICs of 0.8 µg/mL against *P. melaninogenica* and 1.0 µg/mL against A. pyogenes. Additionally, MBC values of AgNPs against *P. melaninogenica and A. pyogenes* were determined to be 1.0 µg/mL and 1.5 µg/mL, respectively (Liao *et al.*,2019). *Klebsiella pneumoniae and Proteus mirabilis* showed inhibition at concentrations of 25 µg/mL, 50 µg/mL, and 75 µg/mL when exposed to morphologically altered AgNPs, with effective elimination occurring at a concentration of 100 µg/mL, as reported by Rajivgandhi *et al.*,2023).

The effectiveness of silver nanoparticles in inhibiting microbial and human cells can be assessed through the measurement of half maximal inhibitory concentration (IC50). For instance, in a study by Manivasagan *et al.*. (2013), HeLa cells were inhibited at concentrations of 200 µg/mL. Another study by Hublikar *et al.*. (2023) found that silver nanoparticles synthesized using Lantana camara leaf extract exhibited an IC50 value of 49.52 g/mL when tested with Cell Proliferation Kit I. At a dose of 50 µg/mL, silver nanoparticles affected mouse embryonic cells, while 1 µg/mL induced cytotoxicity and abnormal cell morphology in the human hepatoma cell line (HepG2), leading to DNA damage, production of reactive oxygen species (ROS), increased oxidative stress, and ultimately apoptosis (Noga *et al.*, 2023). Additionally, synthesized silver nanoparticles demonstrated anticancer activity against Michigan Cancer Foundation breast cell lines (MCF-7) with IC50 values of 2.41 µg·mL−1 (Nguyen *et al.*, 2023). In studies by Bano *et al.*. (2023), the IC50 values of silver nanoparticles for N. meningitidis, S. pneumoniae, and H. influenzae were found to be 82.90, 87.20, and 99.50 µg/ml, respectively. Furthermore, HCT-116 colon carcinoma cells were inhibited at a concentration of 744.23 µg/mL (Dutt *et al.*, 2023).

In experiments involving a mouse model, Alfatemi *et al.*. (2020) determined that the lethal concentration 50 (LC50) of synthesized nonchelating silver nanoparticles sized between 20nm-35nm ranged from 250mg/kg to 350mg/kg. In a study by Mao *et al.*. (2018) using the drosophila model, the range of lethal concentration of silver nanoparticles was observed to start from 20-50µl/ml, with the size of the drosophila decreasing as the concentration of the silver nanoparticles increased.

Table 1: The Minimum Inhibitory and Bactericidal Concentration of AgNPs on Some Nosocomial Pathogens

Some prevalent Nosocomial Pathogen	Forms of AgNPs	AgNPs Size	MIC (µg/Ml)	MBC (µg/ mL)	Ref.
E.coli	Free AgNPs	9.26 to 31.18 nm	85	127.5	(Selem *et al.*,2022)
	Cedecea capped AgNPs	10-100nm	12.5		(More *et al.*,2023b)
	Serum Protein coated AgNPs	2–20 nm	16.0	32.0	(Du *et al.*,2023)
K.pneumoniae	Imipenem-AgNPs Conjugate	20nm	0.015	0.5	(Fontoura *et al.*,2023)
	Amikacin-AgNPs Conjigate	17 nm	3.1	6.9	(Dove *et al.*,2023)
	AgNp	20-65nm	62.5	125	(Siddique *et al.*,2020)
P.aeraginosa	Cedecea capped AgNPs	10-100nm	6.25	12.5	(More *et al.*,2023b)
	Free AgNPs	11nm	15.6	31.25	(Al-Momani *et al.*,2023)
	Free AgNPs	20nm	10.0	15.6	(Abeer Mohammed *et al.*,2022)
E.feacalis	Free AgNPs	40-50nm	2.5	10	(More *et al.*,2023a)
	Free AgNPs	1-100nm	300	300	(Kaukab *et al.*,2023)
	AgNPs – Vancomycin Conjugate	392nm	0.12	1.0	(Veriato *et al.*,2023)
S.aureus	AgNPs – Vancomycin Conjugate	392nm	0.25	1.0	(Veriato *et al.*,2023)
	AgNPs	1-100nm	30	60	(da Cunha *et al.*,2023)

Minimum Cytotoxic Concentration of Silver Nanoparticles

In in-vitro cell culture experiments, it has been observed that silver nanoparticles (AgNPs) can exhibit toxicity towards various human cell lines (Shi *et al.*,2018). While silver nanoparticles (AgNPs) possess various biological properties with extensive applications, our knowledge regarding their potential effect on human health and the environment remains incomplete. The safety of administering AgNPs, whether intravenously or intradermally, is not fully comprehended. Consequently, there is an ongoing concern regarding their potential effects on heart, liver, and kidney functions (Olugbodi *et al.*,2023). Extensive research has been conducted on the toxicity of AgNPs in various models, including animals, cells, and humans, primarily due to concerns about their potential bioaccumulation. AgNPs predominantly enter cells through diffusion and endocytosis, whether through inhalation, ingestion, or skin contact, and tend to amass in different organs, including the heart, liver, and kidneys. In the liver, the presence of AgNPs triggers the generation of ROS (Faúndez *et al.*,2023) and oxidative stress, which, in turn, activate inflammation by stimulating the production of chemokines and cytokines. This inflammatory response can further aggravate damage to the liver (Olugbodi *et al.*,2023). Due to this bioaccumulation, even minute concentrations of AgNPs may be cytotoxic to both microbial and human cells. The cytotoxicity caused by AgNPs may vary depending on their concentration, size, and exposure duration, with a notable emphasis on nanoparticles with sizes equal to or less than 10 nanometers as reported by (Liao *et al.*,2019).

Various studies have investigated the effects of silver nanoparticles (AgNPs) on different cell types. In one study by Dilshad et al. (2020), human breast and liver cell lines were exposed to maleic acid capped AgNPs at a concentration of 50µM. Kakian et al. (2024) studied the impact of AgNPs on Proteus mirabilis bacteria and

Fibroblast L929 cells, using a concentration of 7.8 µg/ml. Halkai et al. (2019) examined human gingival fibroblast cells treated with AgNPs at a concentration of 260 µg/ml. Bhatia et al. (2021) investigated the effects of AgNPs on C2C12 skeletal muscle cell line at a concentration of 5.45 µg/ml. Klein et al. (2023) studied human oral fibroblast cell line exposed to AgNPs at a concentration of 0.015 mg/mL. Lastly, Kadir et al. (2024) looked at the impact of AgNPs on African Green Monkey Kidney, Vero cells at a concentration of 500 µg/mL as presented in the Table 2 below:

Table 2: Minimum cytotoxic concentration of silver nanoparticles

Cell type	Forms of AgNp	MCC	References
Human breast and liver cell lines	maleic acid capped AgNps	50µM	(Dilshad et al., 2020)
-Bacteria-*Proteus mirabilis*	AgNPs	7.8 µg/ml	(Kakian et al., 2024)
-Fibroblast L929 cells			
human gingival fibroblast	AgNPs	260 µg/ml	(Halkai et al., 2019)
C2C12 skeletal muscle cell line	AgNPs	5.45 µg/ml	(Bhatia et al., 2021)
human oral fibroblast cell line	AgNPs	0.015 mg/mL.	(Klein et al., 2023)
African Green Monkey Kidney, Vero cells	AgNPs	500 µg/mL	(Kadir et al., 2024)

MCC = Minimum Cytotoxic Concentration

DELIVERY AND TRANSPORTATION OF SILVER NANOPARTICLE

The impact of silver nanoparticles (AgNPs) is contingent on the delivery method, the combination of compounds involved, as well as their intrinsic characteristics like shape and size, which are significantly shaped by the manufacturing process. Keeping these factors into consideration, silver nanoparticles can serve as a transporter and can be delivered coated using other biomolecules. There are various methods that can be used for the delivery of silver nanoparticles, depending on the intended use.

Topical application of AgNPs in Cream, Gels, Spray, and Nanogels

Silver nanoparticles have been extensively studied for their broad-spectrum antimicrobial properties and have been recently integrated into creams and ointments for topical application in wound healing (Paladini & Pollini, 2019). AgNPs can also be delivered topically through bandages, sutures, plasters, and various other creams and ointments (Parveen *et al.*,2018). Recently, AgNPs have been included in hydrogels, sponges, and electrospun nanofibers. An ointment containing Chloroxine-conjugated Silver nanoparticles has shown effectiveness in wound treatment compared to Calendula flower oil verseline (Nandhini *et al.*,2023). Additionally, a silver nanoparticle-based electrospun spray, combined with polycaprolactone/chitosan, has been tested to create a nanofibrous dressing for wound healing (Saghafi *et al.*,2023). Utilizing AgNPs immobilized in other medical sprays can enhance the treatment of topical bacterial infections.

These nanogels are hydrophilic polymer nanoparticles sized between 20 to 200 nm, which are cross-linked. They can be administered orally, topically, vaginally, via ocular routes, or through other methods. Their size and flexible membranes facilitate controlled drug release, enhanced skin penetration, and swelling through diffusion. Importantly, nanogels are highly biocompatible and can effectively deliver a wide array of hydrophilic medications (Mali & Patil, 2023). Other biocompatible compound drug delivery for antimicrobial purposes includes liposomes, niosomes, microemulsion, nano-emulsion, metal nanoparticles, polymeric nanoparticles, quantum dots, colloidal gold, nano diamonds, dendrimers, nanocrystals, carbon nanotubes (Jayachandran *et al.*,2023).

One of the advantages of topical application of AgNPs, either in composite or conjugate form, is enhanced tissue repair by approximately 99%, achieved through increased angiogenesis, collagen deposition, and accelerated re-epithelialization. The combined action of AgNPs conjugates also leads to an increase in the expression of growth factors and a decrease in the expression of pro-inflammatory factors, particularly IL-6 and TNF-α. These results suggest that with optimized AgNP concentrations, designed antibacterial, cytocompatible, and nanoporous materials can be used as effective wound dressings (Rybka *et al.,* 2022). However, a drawback of these particles is their small size, which allows them to penetrate natural barriers like the skin, potentially resulting in immediate and long-term toxic effects (Ferdous & Nemmar, 2020).

Oral Delivery of AgNPs

For the safe and efficient delivery of silver nanoparticles to host cells, various approaches have been proposed involving the coating, conjugation, and immobilization of AgNPs with different biomolecules. Naturally occurring polymers play multiple roles, such as serving as carriers, providing specific binding surfaces, and offering protective coatings. These polymers can also incorporate biologically active molecules with properties like anti-cancer, antimicrobial, or anticoagulant effects, making them useful as therapeutic agents or natural medications. Polymers derived from plants or microbes, such as poly(saccharides) and poly(phenols), can provide structural support and a means to connect and transport medications (Veerapandian *et al.,*2023). This concept can also be applied to AgNPs. Recent methods for the safe delivery of AgNPs and other nanomaterials include the Red Blood Cell Membrane Camouflaged Nanoparticle (RBCMCN) (Dai *et al.,*2021), originally designed for treating tumor cells but may also be effective against bacteria, the Hyaluronic Acid-based Drug Nano Carrier (HABDNC) (Mauri & Scialla, 2023), the Polymer Lipid Hybrid nanocarrier (PLHN) (de Araujo *et al.,*2023), and various other approaches that can be incorporated into tablets and capsules (Edis *et al.,*2021).

The oral route takes advantage of the highly absorptive intestinal epithelium, which provides a substantial surface area for drug absorption in the gastrointestinal (GI) tract. Every section of the gastrointestinal tract has distinct functions, including protection, secretion, absorption, and digestion (Mathur *et al.,* 2018). Oral administration of silver nanoparticles has several disadvantages, such as the use of preparation techniques that employ toxic organic solvents, which can be harmful to biological systems. Additionally, there is a tendency for the nanoparticles to aggregate and the drugs to be expelled due to crystallization during storage (Lotfipour *et al.,* 2021).

Intravenous Delivery of AgNPs

Using various animal models, intravenous administration of AgNPs has been shown to result in their accumulation in multiple organs (Ferdous & Nemmar, 2020). This method offers a rapid response and precise control over the rate of drug delivery into the body, making it suitable for medications that cannot be absorbed through the gastrointestinal tract or administered via intramuscular or other tissue injections. Importantly, intravenous delivery bypasses first-pass metabolism (Chandrakala *et al.,*2022). However, AgNPs have the potential to disrupt the endothelial barrier by reducing the interaction of VE-cadherin in endothelial cells, leading to increased permeability and subsequent peripheral inflammation in organs like the liver, kidney, and lungs (Guo *et al.,*2016). A study by Wieler *et al.,*(2023) investigated the intravenous application of AgNPs in patients with moderate-severe and severe COVID-19 pneumonia, revealing its safety and efficacy. This approach could serve as a cost-effective and readily available adjunctive therapy for severe COVID-19 pneumonia cases, potentially reducing mortality rates and the need for additional oxygen regardless of the specific SARS-CoV-2 variants. Additionally, AgNPs may hold promise for addressing other respiratory infections, particularly in light of the increasing resistance of pathogens to existing anti-infective agents (Wieler *et al.,*2023).

The advantage of biocompatible AgNPs in intravenous delivery systems allows for precise targeting, resulting in regulated drug release and a sustained impact. These systems offer benefits in cancer treatment, such as improved medication targeting and penetration into tumor cells, enhancing therapeutic efficacy (Nikolova *et al.,* 2023). However, concerns regarding toxicity and immune system recognition pose potential risks that need to be addressed. Extensive research is crucial to improve these systems and overcome their limitations for safe and effective clinical use. Conflicting mechanisms of action, complex engineering processes, high manufacturing costs, and limited practicality for widespread clinical adoption

present additional challenges. Additionally, the potential toxicity of polymeric nanoparticles and their elimination from the body create obstacles for their clinical application (Elumalai *et al.,* 2024).

Nebulized formulation of AgNPs for delivery to the airways

The optimal size of nanoparticles formulated for delivery to the airways ranges between 3-7nm with a minimal bactericidal concentration of 10µg/ml and a calculated tissue deposition concentration of 5µg/ml aerosol droplets as reported by Zachar, (2022). Other trials also utilize 3-7nm AgNPs formulation for airway delivery and a minimum antibacterial concentration of 10-25µg/ml with 5µg/ml aerosol droplets in a cumulative volume of 0.25cc from a source with a concentration of 100ppm (µg/ml) in the bronchial tree, and delivering a total volume of 1cc from a source with a concentration of 250ppm (µg/ml) into the alveoli of the lungs also reported by Zachar, (2020). Silver nanoparticles-OCT mixture encapsulated with alginate to create a carrier called AgNPs-OCT-Alg, designed for drug delivery via nebulization. Each formulation had a total dosage of 1.27 mg/kg, and 50 mL of each formulation was nebulized at a rate of 5 mL/h. In the case of alginate-coated AgNPs-OCT resulting in AgNPs-OCT-Ag, this straightforward delivery carrier was effectively employed for nebulization. The formulation was successfully delivered and internalized in healthy lung tissues through the nebulization method in the animal model (Abdellatif *et al.*,2022).

The nebulized formulation of agnps presents challenges such as rapid drug clearance from the nasal cavity and potential enzymatic degradation, which warrant further investigation. The nasal mucus, nasal epithelium, and lamina propria collectively form the natural anatomical barrier for drug delivery from the nose to the brain. Safety concerns include potential toxicity to nasal cilia or various nasal cells, local inflammation, and systemic effects (Chen *et al.,* 2024). The advantages of intranasal administration of silver nanoparticles include increased loading capacity, protection against degradation, enhanced stability, precise targeting, potential dosage reduction, and the ability to improve affinity for Aß proteins (Dighe *et al.,* 2024).

Intramuscular or subcutaneous injection/delivery of silver nanoparticles

Silver nanoparticles taken orally can be absorbed through paracellular transport, transcytosis, and M-cell uptake within the gastrointestinal tract. Conversely, macrophages and lymphatic uptake primarily absorb nanoparticles through subcutaneous, intramuscular, or inhaled routes (Mahmod & Al-Jumaili, 2022; Mathur *et al.*,2018). The most suitable infection model for evaluating the effectiveness of nanomedicine in animal-based studies to combat infections is a model involving skin and subcutaneous region infections. In this context, silver nanoparticles (AgNPs) were administered intravenously to mice to address subcutaneous abscesses while exposing them to near-infrared (NIR) irradiation. The application of NIR induced a hyperthermal effect, resulting in increased release of Ag and enhanced eradication of MRSA, ultimately promoting the healing of abscesses and wound acceleration (Yeh *et al.*,2020).

The advantages of administering silver nanoparticles intramuscularly include rapid and uniform absorption, allowing for larger drug volumes to be injected compared to subcutaneous delivery. However, there are potential drawbacks to this method, such as injection site discomfort, dosage dependence on muscle mass, the risk of hematoma formation, and the potential for unintentional drug entry into the bloodstream from accidental vessel rupture (Sultana *et al.,* 2022).

STRATEGY FOR SILVER NANOPARTICLE CONJUGATION/IMMOBILIZATION

Many different delivery strategies for AgNPs can assist in safe delivery and prevent the induction of cytotoxicity.

Nanocarriers

Nanocarriers, such as liposomes, micelles, and polymers, can be utilized to encapsulate AgNPs and shield them from degradation and rapid clearance. These nanocarriers offer the advantage of being functionalized to target specific tissues and cells (Tiwari *et al.*,2023). They represent a promising strategy for delivering AgNPs to specific tissues and cells, enhancing their stability, circulation time, and targeting precision. Nandhini *et al.*,(2023)

introduced a novel micellar nanocarrier tailored for agnp delivery to wound sites. This nanocarrier was engineered to release AgNPs gradually, thereby fostering the process of wound healing.

Conjugates

AgNPs can be conjugated to various molecules, including antibodies, peptides, and aptamers, to target specific receptors and transporter proteins (Todorova *et al.*,2023). One significant benefit of AgNP conjugates is their ability to target specific cells or tissues by attaching them to molecules that bind to specific receptors on the target cells or tissues. For instance, AgNPs can be linked to antibodies that target cancer cells or peptides that target bacteria (Veerapandian *et al.*,2023).

Another advance in nanoparticle conjugation involves the immobilization of silver nanoparticles using enzymes or as capping enzymes. The conjugation of silver nanoparticles with lysozyme and colistin was proven effective in disrupting bacterial biofilms (Meesaragandla *et al.*,2022). Additionally, the synergistic conjugation of lysozyme with AgNPs increased the antibacterial activity of the conjugates by 86% (Ernest *et al.*,2014).

External stimuli

External stimuli, such as magnetic fields, ultrasound, and light, can be utilized to regulate the release of AgNPs from nanocarriers or conjugates (Jain *et al.*,2022). The development of external stimuli-responsive AgNPs has addressed this challenge by enabling the controlled release of drugs in response to specific stimuli like light, magnetic fields, and ultrasound (Kah *et al.*,2023). This tailored approach facilitates precise and targeted drug delivery to distinct cells and tissues. For example, light-responsive AgNPs have been engineered to release their drug payload upon exposure to near-infrared light, proving effective in delivering drugs to tumors in mice with minimal side effects. Similarly, magnetic-responsive AgNPs have been devised to release drugs in response to an alternating magnetic field, demonstrating efficacy in drug delivery to the brain in rats while minimizing damage to healthy tissue (Sahoo *et al.*,2023).

CONCLUSION

Effective AgNP antimicrobial activity in the treatment of pathogens, including covirulent multidrug and hypervirulent strains, depends on the delivery method, conjugates, and nanocarriers. The stepwise mechanism of AgNPs' actions and recent advances in delivery methods provide a roadmap for the safe delivery of AgNPs as alternative nanobiotics for treating resistant strains. Various forms of AgNPs in conjugates and capped forms, when applied in sprays and antiseptics, have been shown to be effective against nosocomial pathogens, thus contributing to the prevention of hospital-acquired infections and, consequently, community-acquired infections.

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CONFLICT OF INTEREST

No conflict-of-interest record about this study

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