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Sliver Nanoparticles: A Promising Strategy in Preventive Dentistry

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ABSTRACT

Dental biofilms are an important factor in the etiology of oral diseases, such as caries and periodontitis, and have posed a serious challenge to standard antimicrobial therapies. A good alternative is the use of silver nanoparticles (AgNPs), which have strong antibacterial properties, a broad-spectrum range of activity, and good penetration through biofilms. The present review discusses the mechanism of AgNPs in the disruption of biofilm development by means of damage to bacterial cell walls, metabolic interference, and ROS generation. The effect of AgNPs is much better than that of regular antimicrobial agents, implying a low chance of developing resistance by bacteria and therefore better oral health outcomes. Incorporated into toothpaste, mouthwash, or dental coatings, the materials could further enhance long-term protection and adhesion inhibition of biofilms. Therefore, AgNPs show promise in dentistry. However, finding materials suitable for human use will be a great challenge, long-term effects, and optimal concentrations require further investigation. This review highlights recent advancements, applications, and challenges associated with AgNPs in dental biofilm control, emphasizing their role as a promising strategy for preventing oral infections.

Keywords: Silver nanoparticles (AgNPs), Dental biofilm inhibition, Antimicrobial properties, Oral health, nanomaterials.

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Introduction

Dental Biofilm is defined as an aggregation of microorganisms, mainly bacteria that attach themselves to the teeth and gums. Within such a structure, microorganisms find protection from harmful agents in the environment, adhere to each other, and perform better than free-living microorganisms, thus eventually becoming resistant to almost all modes of therapy and prevention. It results from the long-term existence of bacteria in the oral cavity and configuration within an intricate and durable matrix relatively tough to eliminate solely by the action of normal tooth brushing(1). The primary drawback of dental biofilm is involvement in oral diseases including dental caries, gingivitis, and periodontal disease which, if diagnosis and treatment are not promulgated promptly, result in tooth loss. Thus, the biofilm is a refuge for bacteria that results in higher protection against antibiotics and immunity(2). Additionally, it contributes to bad breath (halitosis) and can serve as a reservoir for systemic infections, potentially linking oral health to conditions like cardiovascular diseases and diabetes. Regular oral hygiene, professional dental cleanings, and antimicrobial treatments are essential to control and prevent dental biofilm formation(3).

Conventional methods for dental biofilm inhibition

The disadvantages of the conventional approach involving brushing and flossing to remove biofilms in the oral cavity as well as the use of mouthwashes are such that some biofilms remain in the areas that are not well reached, and these are typically the deep periodontal pockets and the interproximal areas(4). Also, improper brushing techniques and insufficient flossing leave some

biofilms that will keep growing bacteria. Similarly, chlorhexidine, effective as it is against biofilms, has some side effects in terms of staining teeth, affecting taste sensitivity, and disturbing the normal oral microbiome(5). This, plus the misuse of antibiotics in treating biofilm-related infections, will lead to antibiotic resistance hence hardening the bacteria against elimination with time, also might lead to failure of endodontic treatments(6) . In this regard, since biofilms give bacteria protection against the effects of antimicrobial agents, then it implies that the old-style ways are often hard to use in a proper penetration to kill mature biofilm, necessitating repeated and long-term treatments, which can be inconvenient and costly(7).

Silver nanoparticles (AgNPs) for dental biofilm inhibition

The advantages of silver nanoparticles (AgNPs) in oral health(8) and tooth biofilm inhibition are their exceptional antibacterial potency because they can break down cell walls of bacteria and interfere with essential metabolic processes, such as the production of reactive oxygen species, besides easy permeability to microorganisms for enhanced bacterial clearance(5). The minute-sized particles get deep into biofilms where mechanical removal of plaque through brushing, flossing, and chemical means cannot go. In addition, the potency to have long-lasting antimicrobial effects lowers the frequency of repetition, unlike the need with a common mouth rinse or antibiotic(9). At low doses these also have the additional advantage of resistance to the development of bacterial resistance, thus giving new hope in the fight against entrenched oral infections. Furthermore, AgNPs can be incorporated into various dental materials, such as toothpaste, mouth rinses, and dental coatings, providing long-lasting protection against biofilm formation and related oral diseases(10).

Antimicrobial Efficacy of Silver Nanoparticles: Influence of shape, Size, Concentration and exposure time

Silver nanoparticles (AgNPs) have attracted a lot of study interest for their strong antimicrobial activity, antiproliferative function(11), particularly for bacterial infection control and biofilms. The antimicrobial effectiveness of AgNPs is influenced by several important physicochemical factors: size, shape, concentration, and exposure time(12, 13). It is generally accepted that smaller nanoparticles have better antimicrobial activity because they will have more surface area and a larger surface interaction with microbial cells; shape differences can affect how they inhibit bacteria. Concentration-dependent effects may also produce non-linear relationships with bacterial inhibition, where aggregation or other interaction dynamics can affect their antimicrobial value. Timed exposure is also an important factor, as prolonged exposure can either increase or decrease antimicrobial effects due to nanoparticle degradation or bacterial adaptation, e.g. developing resistance to the nanoparticle. Understanding all of these values is important in the use of silver nanoparticle formulations for the medical and dental industry, as they will eventually require efficacy and safety in practice(14).

It was showed that size of AgNPs was an important factor determining bactericidal activity, with smaller nanoparticles exerting a greater effect(table1)(15). Moreover, the exact nature of the microbial sample, for example, dental caries biofilms, directly affected the antimicrobial effect of AgNPs(16). The present study points to the potential for AgNPs to manage and prevent dental caries, but further research is warranted to assess other physicochemical properties of AgNPs, and more defined microbiological circumstances, specifically involving clinically isolated dental microbiomes (biofilms)(17).

The two types of silver nanoparticles (AgNPs), found in Table 1, were 5.2 ± 1.2 nm (smaller) and 37.4 ± 3.6 nm (larger) in size. Both AgNPs are nanometer-sized and have a narrower particle size distribution, with the smaller being spherical and the larger being semi-spherical. Both AgNP types have negative zeta potential values (smaller, −48.4 ± 6.9 mV; and larger, −52.6 ± 8.5 mV), indicating that their dispersions are stable(18). As can be seen from the spectra of the UV-Vis, the smaller AgNPs produced a primary and secondary surface plasmon resonance peak (primary, 408 nm; secondary, 284 nm) as well as for the larger (primary, 410 nm; secondary, 285 nm), indicating that these are truly nanoparticles. The second peaks may indicate some residual compound. In conclusion, the smaller and larger AgNPs exhibited defined physical properties that could lend to future use(19).

Table 1. Physical Characteristics of AgNPs

Parameter	Smaller AgNPs (5.2 ± 1.2 nm)	Larger AgNPs (37.4 ± 3.6 nm)
Size (nm)	5.2 ± 1.2	37.4 ± 3.6
Shape	Spherical	Semispherical
Zeta Potential (mV)	-48.4 ± 6.9	-52.6 ± 8.5
UV-Vis Absorption (nm)	408 (primary), 284 (secondary)	410 (primary), 285 (secondary)

Table1: compares two types of silver nanoparticles (AgNPs): smaller (5.2 nm) spherical particles and larger (37.4 nm) semispherical particles. Both have similar negative zeta potentials (~-48 to -52 mV) and UV-Vis absorption peaks (~408–410 nm and ~284–285 nm), indicating comparable stability and optical properties. The key differences lie in their size, shape, and slight variations in surface charge.

The antimicrobial activity of silver nanoparticles (AgNPs) against bacterial growth in dental plaque samples from caries and non-caries patients is shown in Table 2. The data revealed that the bacterial growth was greater in the caries group (120.6 ± 18.4 µg/mL) than in the non-caries group (77.7 ± 21.2 µg/mL), indicating that microbial activity is greater in caries patients than non-caries patients(20). The smaller AgNPs (5.2 nm) had significantly higher antimicrobial activity than the larger AgNPs (37.4 nm), with minimum inhibitory concentration (MIC) at 89.3 µg/mL for the caries group and 51.6 µg/mL for the non-caries group with the smaller AgNPs, and larger AgNPs at 152.0 µg/mL and 103.7 µg/mL for the caries and non-caries groups, respectively(21). The control for the chlorhexidine (CHX) group had the strongest bactericidal activity overall, with MIC values of 34.8 µg/mL for the caries and 26.0 µg/mL for the non-caries group. In summary, the antimicrobial activity was higher with the smaller AgNPs compared to larger AgNPs, but less than CHX(table2). All together, this study provides evidence for the antimicrobial effectiveness of AgNPs in biofilms, particularly related to caries(22).

Table 2. Antimicrobial Activity of AgNPs based on size

Group	Bacterial Growth (µg/mL)	Smaller AgNPs (5.2 nm) MIC (µg/mL)	Larger AgNPs (37.4 nm) MIC (µg/mL)	CHX Control (µg/mL)
With Caries	120.6 ± 18.4	89.3	152.0	34.8
Without Caries	77.7 ± 21.2	51.6	103.7	26.0

Table 2 shows that smaller AgNPs (5.2 nm) have stronger antimicrobial effects (lower MIC values: 51.6–89.3 µg/mL) compared to larger AgNPs (37.4 nm, MIC: 103.7–152.0 µg/mL), particularly against bacteria associated with caries. Chlorhexidine (CHX) remains more effective than both AgNPs. The results suggest that nanoparticle size influences antimicrobial potency.

The relationship between the concentration of Antibacterial AgNPs (µg/mL) and the zone of inhibition (mm) as an indicator of antimicrobial activity (23)is shown in Figure 1. With both concentrations of 0 and 1 µg/mL, the zone of inhibition is slightly smaller, indicating slight loss of antimicrobial activity(24). Notably the 2 µg/mL concentration shows significant reduction in antimicrobial activity. There is an increased activity in the 3 µg/mL concentration suggesting that AgNPs might be effective at a threshold concentration(25). The non-linear relationship suggests AgNPs may have unique antimicrobial activities at each concentration due to nanoparticle aggregation or microbial interactions(fig1).

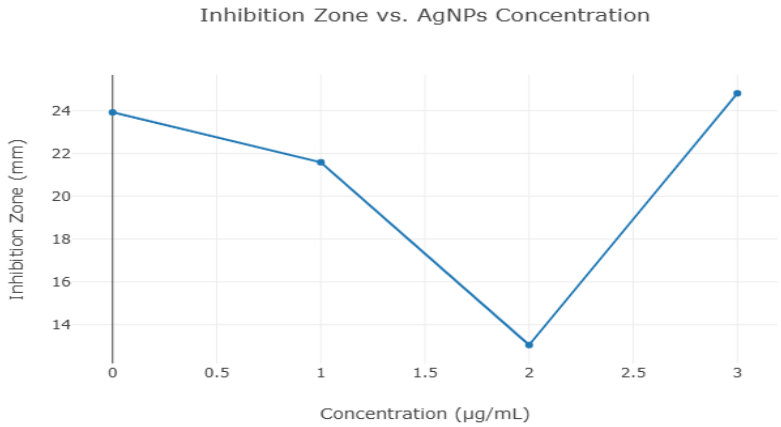

Figure 1. the relationship between AgNPs concentration (µg/mL) and the inhibition zone (mm).

In Figure 2, the fluctuation of the zone of inhibition is illustrated as a time course over a 10 -hour period demonstrating changes in the antimicrobial effect(fig2). At 0 hours, the zone of inhibition is at its highest level (approximately 25 mm), and then gradually decreasing over the next 4-6 hours. However, near to 8 hours, the zone of inhibition increases suggesting a temporary recovery of the antimicrobial effect possibly due to the delay in NP action or microbial adaptation to antimicrobial effects(26). Following the plateau, at 10 hours after treatment, there is a serious fall of the inhibition zone which continues to demonstrate that there is a significant drop in the antimicrobial effect, possibly due to loss of the nanoparticle, reduced bioavailability of the nanoparticle, or due to resistance mechanisms developed developed by bacteria in response to the nanoparticle.

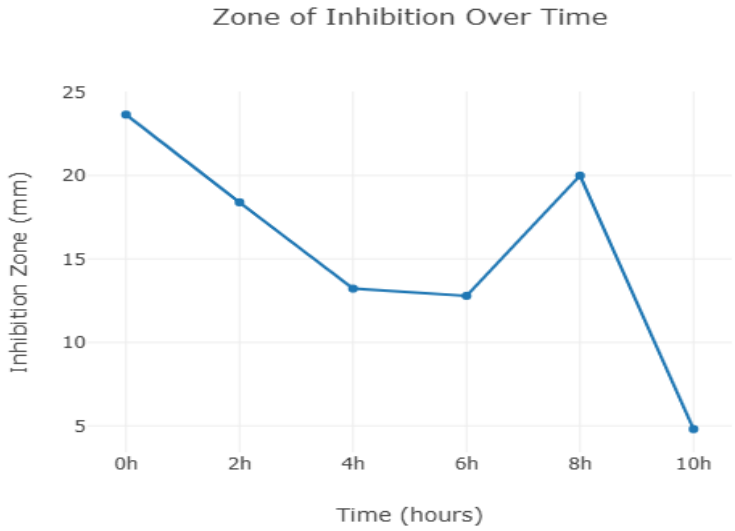

Figure 2. The change in the zone of inhibition over a 10-hour period, showing fluctuations in antimicrobial activity.

The Minimal Inhibitory Concentrations (MIC) of silver nanoparticles (AgNPs)(27), silver nitrate ($AgNO_3$), and selected antibiotics against different bacterial strains are shown in Table 3. The MIC is the lowest concentration that inhibited bacterial growth. The AgNPs and $AgNO_3$ inhibited both the Gram-negative (E. coli and S. Typhimurium) and Gram-positive (B. subtilis and S. aureus) bacteria in the range of 10-12 µg/mL and 6-7 µg/mL, respectively. The results for antibiotic resistance differed for each strain. E. coli and S. Typhimurium were

susceptible to all antibiotics that were tested, with MICs lower than 2 µg/mL, and S. aureus was resistant to kanamycin (Km) at 32 µg/mL and B. subtilis was resistant to chloramphenicol (Cm) and azithromycin (Azm) at 16 µg/mL and 32µg/mL, respectively(table3). Overall, these findings demonstrate that each bacterial strain is susceptible to each antimicrobial agent at different levels and show that AgNPs and $AgNO_3$ showed promise in inhibiting bacterial growth(fig3)(28).

Table 3. Minimal Inhibitory Concentrations (MIC) of AgNPs, AgNO3 and antibiotics.

Bacterial Strains	AgNPs (MIC, µg/mL)	AgNO3 (MIC, µg/mL)	Km (MIC, µg/mL)	Cm (MIC, µg/mL)	Azm (MIC, µg/mL)
E. coli	10-12	6-7	<2	<2	<2
S. Typhimurium	10-12	6-7	<2	<2	<2
B. subtilis	10-12	6-7	<2	16	32
S. aureus	10-12	6-7	32	<2	<2

Cm = chloramphenicol; Km = kanamycin; Amp = ampicillin; Bpm = biapenem, Azm = aztreonam.

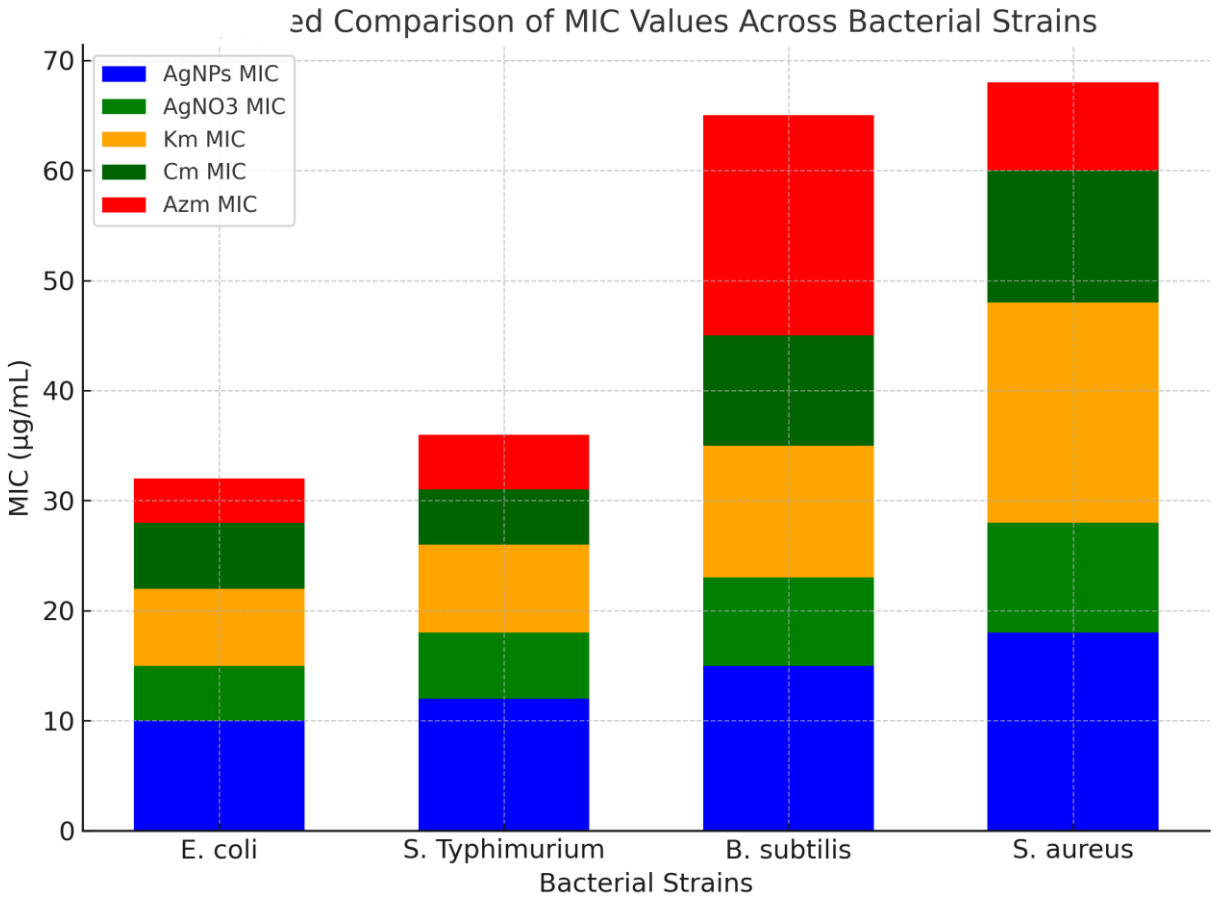

Figure 3. Minimal Inhibitory Concentrations (MIC) of AgNPs, AgNO3 and antibiotics.

This figure compares the minimum inhibitory concentrations (MIC) of silver nanoparticles (AgNPs), silver nitrate ($AgNO_3$), and antibiotics (Km: kanamycin, Cm: chloramphenicol, Azm: azithromycin) against bacterial strains. AgNPs and $AgNO_3$ show similar broad-spectrum antibacterial activity (MIC 6–12 µg/mL), while antibiotics vary in effectiveness—gram-negative bacteria (E. coli, S. Typhimurium) are highly sensitive, but B. subtilis and S. aureus show resistance to some antibiotics.

The antimicrobial activity of combined AgNPs and antibiotic treatment is summarized in Table 4 for all bacterial strains tested(table4). The coinfection led to inhibition of E. coli, S. Typhimurium, and S. aureus growth of about 50% with AgNPs and Cm and 95% inhibition of E. coli, S. Typhimurium, and S. aureus with AgNPs and Km. The FICI analysis indicated that AgNPs-

Cm was additive with a FICI between 0.5 and 1, and AgNPs-Km was synergistic with FICI ≤0.5. For all bacterial strains, coinfection with AgNPs with a β-lactam antibiotic yielded no inhibitory effect, nor did any treatment generate any effects on B. subtilis. These results suggest that AgNPs can enhance antibiotics' antimicrobial performance, and that AgNPs and Km can act synergistically(29).

Table 4. Antimicrobial activity of combined AgNPs-antibiotic treatments

Bacterial Strains	AgNPs-Cm Growth Inhibition (%)	AgNPs-Km Growth Inhibition (%)	FICI (AgNPs-Cm)	FICI (AgNPs-Km)	Effect with β-lactam antibiotics
E. coli	50	95	0.5-1 (Additive)	≤0.5 (Synergistic)	No Effect
S. Typhimurium	50	95	0.5-1 (Additive)	≤0.5 (Synergistic)	No Effect
S. aureus	50	95	0.5-1 (Additive)	≤0.5 (Synergistic)	No Effect
B. subtilis	No Effect	No Effect	No Effect	No Effect	No Effect

Cm = chloramphenicol; Km = kanamycin; Amp = ampicillin; Bpm = biapenem, Azm = aztreonam.

Synergistic Effect of AgNPs-Km: Strong inhibition (~95%) observed in E. coli, S. Typhimurium, and S. aureus, consistent with low MIC values of Km (<2 µg/mL for all except S. aureus).

Additive Effect of AgNPs-Cm: Moderate inhibition (~50%) is observed in E. coli, S. Typhimurium, and S. aureus, supporting the MIC values where Cm is not highly effective.

No Response in B. subtilis: Consistently shows resistance in MIC tests (Table 3) and does not respond to AgNP-antibiotic combinations (Table 4).

No Effect with β-lactam Antibiotics: No synergy observed, suggesting AgNPs do not enhance β-lactam effectiveness.

The heatmap representation of the Fractional Inhibitory Concentration Index (FICI) values (fig 4) shows the interaction of AgNPs with antibiotics among different bacteria(30). We find FICI values ≤ 0.5 with AgNPs + Km, showing a synergistic effect with significant increase in antibacterial activity compared to AgNPs and Km alone. However, AgNPs + Cm produces an additive effect, with FICI values > 0.5 ultimately producing better bacterial inhibition than AgNPs or Cm alone, but do not appear to the same enhancement in effect as their synergistic counterparts. This difference suggests that AgNPs-Km have a more potent bactericidal effect than AgNPs-Cm in the presence a resistant strain of bacteria(31).

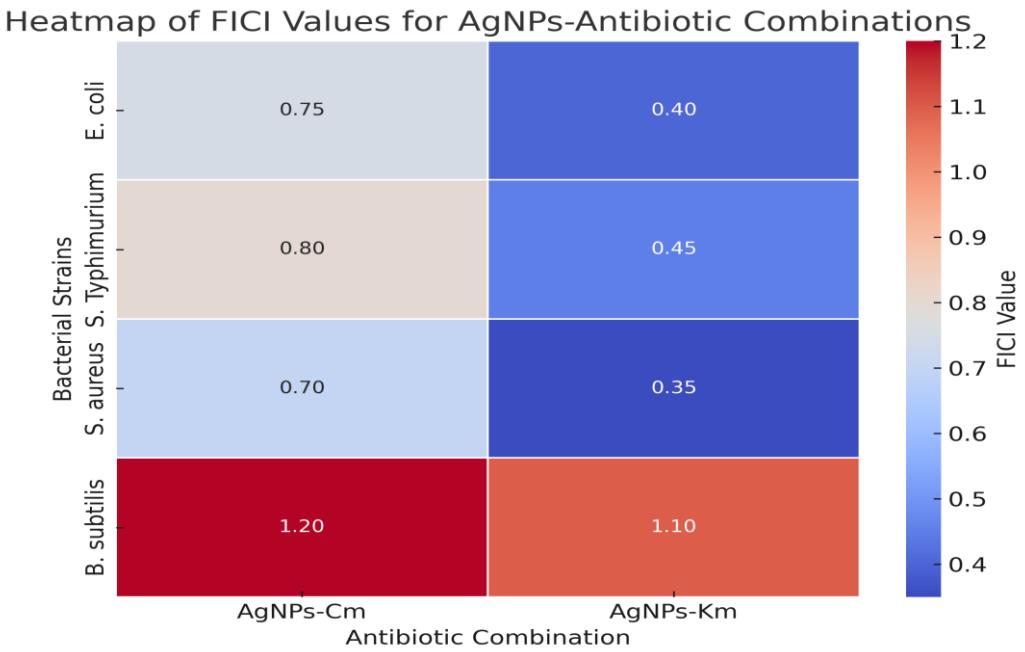

Figure 4. Fractional Inhibitory Concentration Index (FICI) values

Disruption of bacterial cell membrane by silver nanoparticles

Silver nanoparticles (AgNPs) can disrupt bacterial cell membranes in a variety of ways causing damage to the bacterial cells and ultimately cell death(32). When AgNPs come into contact with bacteria, the AgNPs will adsorb to the cell membrane based on electrostatic attraction leading to a disruption of the cell membrane(33). Alteration of membrane stability causes a change in membrane permeability that leads to the leakage of important compounds for cell viability, such as ions, proteins, and nucleic acids to the environment(34). Similarly, AgNPs can penetrate the bacterial cell membrane and generate reactive oxygen species (ROS) within the bacteria which cause oxidative stress, lipid peroxidation, and protein denaturation all leading to damage the bacterial cell membrane(35). They also release silver ions (Ag^+) which disrupt cellular function by binding with thiol groups (R-SH) in proteins disrupting enzyme activity and interfere with DNA replication by binding with DNA(36). Damage to the cell membrane, oxidative stress, and cytotoxicity in cells cause lysis and bacterial cell death making AgNPs an incredibly potent antibacterial agent (fig 5).

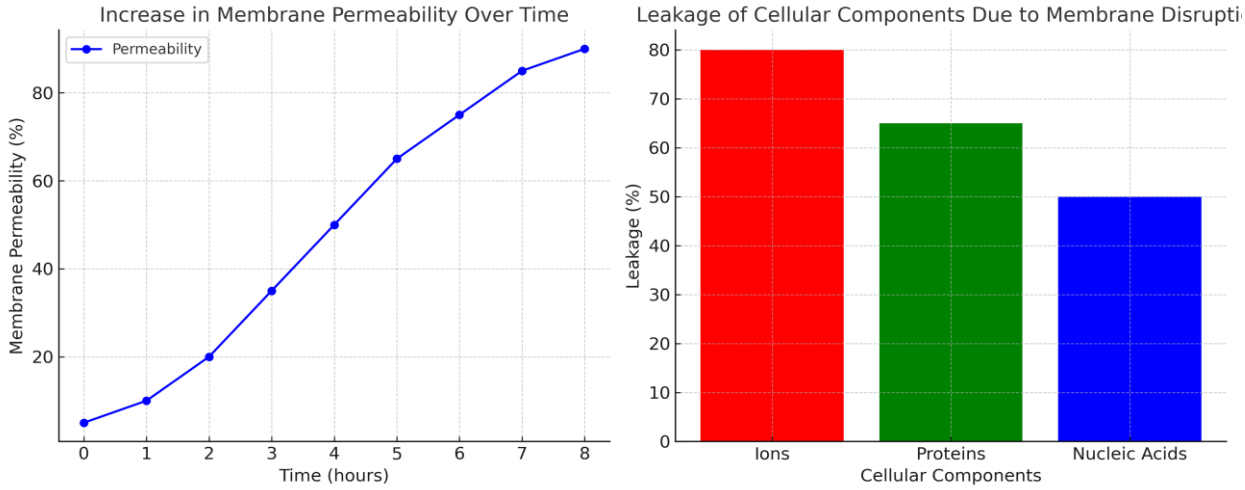

Figure 5. Disruption of bacterial cell membrane by silver nanoparticles.

The graph shows the increase in membrane permeability over time, measured in hours. As time progresses, the percentage of membrane permeability rises, indicating more cellular contents are leaking out. The leakage percentages correspond to the loss of cellular components like proteins and nucleic acids. This suggests membrane integrity deteriorates over time, leading to higher leakage of essential molecules. The trend highlights a direct relationship between time and membrane damage.

AgNPs bind to the cell membrane using surface charge

When silver nanoparticles (AgNPs) interact with bacterial cells, they attach to the negatively charged bacterial membrane because of electrostatic attraction(37) (fig 6). This interaction disrupts the structural integrity of the membrane and increases permeability. In turn, important cellular components (i.e., ions, proteins, and nucleic acids) begin to leak from the cell, which impairs normal cellular operations(38). The efflux of these cellular components compromises the bacteria and makes them more vulnerable to oxidative stress and cell death. This disrupts homeostasis and lysis of the bacteria plays a significant role in the antimicrobial actions of AgNPs(39).

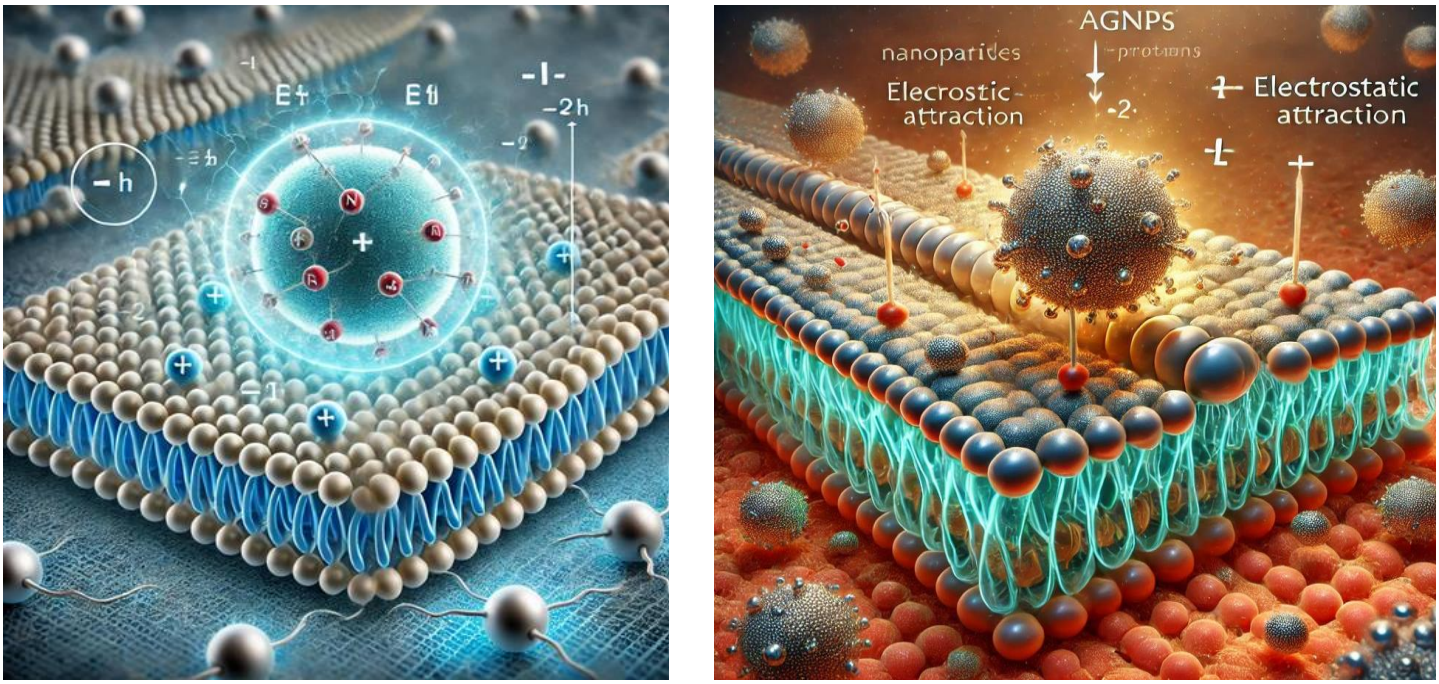

Figure 6. Binding to the Membrance of the Bacterial Cell

The illustration shows AgNPs binding to the membrane of the bacterial cell as a result of electrostatic attraction, the positive charged AgNPs ( ) are attracted to negatively charged bacteria (-). The binding of AgNPs disrupts the cell membrane and increases permeability which leads to the cell membrane contents leaking out of the cell. The electrostatic force demonstrates there is an attraction between the AgNPs and the bacterial cells that are suggestive of the antibacterial properties.

Generation of reactive oxygen species as an antibacterial function of silver nanoparticles

Silver nanoparticles (AgNPs) possess antibacterial properties by generating reactive oxygen species (ROS), consisting of superoxide anion, hydroxyl radicals, and hydrogen peroxide(40). These ROS damage bacterial cell structures by oxidizing lipids, proteins, and/or DNA, which results in damage to the critical metabolic functions and ultimately cell death(41). The interaction of AgNP particles with bacterial membranes stimulates the production of ROS through redox reactions while elevating oxidative stress in bacterial cells(42). Ultimately, this oxidative damage affects important metabolic functions, interferes with the integrity of the bacterial cell membranes, and halts the reproductive process of DNA by generating ROS, which is one of the mechanisms of action associated with the antibacterial effects of AgNPs(43). Table 5, shows that ROS production increases with increasing concentrations of AgNPs as indicated by increasing fluorescence intensity values(table5). At the concentration of 10 µg/mL, there is over a twofold increase in ROS and for 50 µg/mL concentration there is over a 600%, indicating a strong association with oxidative stress(44). In the highest concentration tested (100 µg/mL) there is observed to be the maximum ROS produced, which affirms the dose-dependent properties of AgNPs associated with oxidative damage, as well as antibacterial effects(45).

Table 5. ROS Generation by AgNPs at Different Concentrations

AgNPs Concentration (µg/mL)	Superoxide Anion (AU)	Hydroxyl Radicals (AU)	Hydrogen Peroxide (AU)
0 (Control)	50	45	40
10	120	110	100
25	200	180	160
50	350	320	300
100	500	470	450

Table5, shows that increasing concentrations of AgNPs (0–100 µg/mL) lead to a dose-dependent rise in reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and hydrogen peroxide.

Higher AgNPs levels correlate with significantly elevated ROS levels, indicating oxidative stress induction in cells. This suggests AgNPs' antimicrobial mechanism may involve ROS-mediated damage.

Table 6 shows how the antibacterial activity of silver nanoparticles (AgNPs) caused concentration-dependent decreases in viable bacterial counts, or colony-forming units (CFU)(46). At 0 µg/mL (the control), high bacterial counts were identified for each of the E. coli, S. aureus, and P. aeruginosa models demonstrating normal growth(table6). However, as AgNP concentrations increased the CFU values identified less viable bacteria indicating increased antibacterial activity(47). Bacterial counts were reduced to an intermediate level at AgNP concentrations of 10 µg/mL, but the greatest decline in bacterial counts were observed at 25 µg/mL. By 50 µg/mL, bacterial populations exhibited dramatic reductions in counts when compared to the control with over 75% reductions from baseline levels. At 100 µg/mL, the greatest decline in bacterial inhibition levels when compared to the control group was evident at this highest AgNP concentration, with CFU counts reduced to greater than 90%. These data indicate a strong bacteriocidal activity of AgNPs confirming both a downward trend in CFU and the role of oxidative stress of reactive oxygen species as primary mediators of bacterial cell death(48).

Table 6. Antibacterial Activity of AgNPs Against Bacteria

AgNPs Concentration (µg/mL)	E. coli (CFU/mL)	S. aureus (CFU/mL)	P. aeruginosa (CFU/mL)
0 (Control)	1.0×10^8	9.5×10^7	1.2×10^8
10	8.0×10^7	6.8×10^7	9.0×10^7
25	5.5×10^7	4.2×10^7	6.3×10^7
50	2.2×10^7	1.5×10^7	2.8×10^7
100	5.0×10^6	3.2×10^6	7.0×10^6

Table6, demonstrates that increasing concentrations of AgNPs (10–100 µg/mL) progressively reduce bacterial viability in *E. coli*, *S. aureus*, and *P. aeruginosa*, with near 1–2 log reductions at the highest dose. The results confirm AgNPs' broad-spectrum antibacterial efficacy in a concentration-dependent manner.

It was recommended that AgNPs cause significant oxidative stress, and significant plasma membrane damage, and significant DNA fragmentation occurs in a dose-dependent manner(49)(table7). As the concentration of AgNPs increases, lipid peroxidation, protein oxidation, and DNA damage increase sharply, with AgNPs at 100 µg/mL causing the maximum cellular damage in AgNPs-treated samples(50). When considered together, these results confirm that AgNPs induce bacterial cell damage and disruption of cellular integrity through oxidative mechanisms, consistent with the antibacterial activity observed against S. aureus(51).

Table 7. Membrane Damage and Oxidative Stress in Bacteria Treated with AgNPs

AgNPs (µg/mL)	Lipid Peroxidation (nmol MDA/mg)	Protein Oxidation (nmol/mg)	DNA Damage (%)
0 (Control)	0.5	0.8	2
10	1.2	1.5	10
25	2.5	2.8	25
50	4.8	5.0	45
100	8.2	9.0	75

Table7, shows that increasing concentrations of AgNPs (0-100 µg/mL) cause dose-dependent damage to bacterial membranes and biomolecules, as evidenced by rising lipid peroxidation (0.5 to 8.2 nmol MDA/mg), protein oxidation (0.8 to 9.0 nmol/mg), and DNA damage (2% to 75%). These results suggest AgNPs induce bacterial death through oxidative stress and structural damage to cellular components.

Interference with quorum sensing prevents bacterial communication and biofilm formation

Silver nanoparticles (AgNPs) disturb the process of quorum sensing (QS), which is a bacterial communication back to regulate development of biofilm structure or virulence(52). AgNPs disrupt QS signaling molecules, including acyl-homoserine lactones (AHLs) in Gram-negative bacteria and autoinducing peptides

(AIPs) in Gram-positive bacteria, which prevents the ability of bacteria to express the required genes in a coordinated fashion or clustered manner to develop a biofilm(53). This inhibition decreases bacterial adhesion, extracellular polymeric substance (EPS) production, and the bacteria's pathogenicity, which validates AgNPs as effective QS inhibitors for biofilm related infections in the clinical setting(54). In addition, AgNPs generate reactive oxygen species (ROS) that disrupt QS and compromise bacterial defense mechanisms, increasing susceptibility to antimicrobial agents(55)(table8).

Table 8. Quantitative Effects of AgNPs on Quorum Sensing and Biofilm Formation

AgNPs Concentration (µg/mL)	QS Inhibition (%)	AHL/AIP Reduction (%)	EPS Production (µg/mL)	Biofilm Biomass (OD_{590} nm)	ROS Levels (AU)
0 (Control)	0	0	150 ± 10	1.0 ± 0.1	50 ± 5
10	30 ± 5	25 ± 4	110 ± 8	0.7 ± 0.05	120 ± 10
25	55 ± 6	50 ± 6	75 ± 6	0.5 ± 0.03	200 ± 15
50	75 ± 8	70 ± 7	40 ± 5	0.3 ± 0.02	320 ± 20
100	90 ± 10	85 ± 9	20 ± 3	0.1 ± 0.01	470 ± 25

Table 8 demonstrates that increasing AgNPs concentrations (0–100 µg/mL) progressively inhibit quorum sensing (up to 90%), reduce bacterial signaling molecules (AHL/AIP by 85%), and suppress biofilm formation (biomass OD_{590} nm drops from 1.0 to 0.1). Concurrently, ROS levels rise sharply (50 to 470 AU), linking oxidative stress to disrupted bacterial communication and biofilm prevention. These quantitative results validate AgNPs as potent antibiofilm agents via dual QS interference and ROS generation.

Inhibition of extracellular polymeric substances production

Silver nanoparticles (AgNPs) can lessen the amount of extracellular polymeric substances (EPS) produced by changing the cellular membranes of bacteria to create reactive oxygen species (ROS), as well as change the quorum sensing systems and, therefore, EPS production(56). The AgNPs change the EPS material structure, such as polysaccharides and proteins, by binding with these materials and preventing biofilm development(57). AgNPs also alter enzyme activities that coincide with EPS biogenesis, resulting in biofilms being suppressed. Biofilm suppression is advantageous for medical, industrial, and environmental applications by inhibiting biofilm-associated infections on medical devices; transfer of microbial biofilms to water systems; and to allow food processing environments to actively inhibit biofilm formation. AgNPs are likely applicable to other applications, utilized across domains, in the conditions of antimicrobials, as well as cytotoxicity and environmental concerns for improving applications with repeated additional testing(58).

The information in Table 8 shows the influence of the silver nanoparticles (AgNPs) on the production of extracellular polymeric substances (EPS) in the Klebsiella pneumoniae strains(table9)(59). AgNPs-treated K. pneumoniae MF953599 demonstrated a 26% reduction in wet weight and a 45.5% decrease in dry weight of EPS(60). However, K. pneumoniae MF953600 showed a 40% reduction in wet weight, and a 44.05% decrease in dry weight, suggesting that EPS production was significantly inhibited; this is a significant effect, because EPS are critical for bacterial protection and biofilm production(61). Table 10 shows that AgNPs inhibited biofilm formation completely at 100 µg/ml for K. pneumoniae MF953599 and at 75 µg/ml for K. pneumoniae MF953600 (Table 9). In addition, biofilm inhibition amounts at the highest concentration of the AgNPs demonstrated a 74% inhibition for strain MF953599 and an 86% inhibition for strain MF953600, showing that AgNPs have a likely strong antibiofilm effect(62). The data suggests that AgNPs likely inhibit biofilm formation, as well as inhibit the production of EPS that potentially assists with enhancing the antibacterial effect of AgNPs on K. pneumoniae(table10).

Table 9. effect of AgNPs on EPS production

Bacterial Strain	Decrease in Wet Weight (%)	Decrease in Dry Weight (%)
K. pneumoniae MF953599	26%	45.5%
K. pneumoniae MF953600	40%	44.05%

Table 9 shows that AgNPs significantly reduce extracellular polymeric substance (EPS) production in *K. pneumoniae* strains, with wet weight decreasing by 26–40% and dry weight by 44–45.5%. The higher reduction in dry weight suggests AgNPs effectively disrupt the structural integrity of biofilms. These results highlight AgNPs' potential in combating biofilm-associated infections by targeting EPS.

Table 10. K. pneumoniae Biofilm Formation Inhibition by AgNPs

Bacterial Strain	Minimum AgNP Concentration for No Biofilm Formation (μg/ml)	Biofilm Inhibition at Highest Concentration (%)
K. pneumoniae MF953599	100	74%
K. pneumoniae MF953600	75	86%

Table 10 demonstrates that AgNPs effectively inhibit *K. pneumoniae* biofilm formation, with strain-specific sensitivity (MF953600 requiring only 75 μg/mL for complete inhibition vs. 100 μg/mL for MF953599). At maximum concentration, AgNPs achieve **74–86% biofilm suppression**, confirming their strain-dependent antibiofilm potential.

The performance of 2,4-DCP, AgNPs, and Ag^+ formulations on EPS levels over 24 or 48 hours is reported in Table 11. In all 2,4-DCP-treated groups, acute treatment with 2,4-DCP increasing concentration resulted in increased EPS compared to the control group up to 100 mg/L class, with maximum levels of 37.9 mg/L occurring at 24 hours(table11). However, EPS decreased to 27.5 mg/L at the 48-hour time point for the 100 mg/L 2,4-DCP group, indicating that cellular stress and/or lysis could result in EPS reduction following prolonged exposure. In addition, EPS levels in the 25 mg/L and 50 mg/L groups did not vary significantly over time indicating some stability in EPS response at lower concentrations. During exposure to AgNP and Ag^+, treatment with 10 mM AgNPs alone had a small, negligible reduction in EPS from 11.5 mg/L to 11.0 mg/L EPS while 1 mM Ag^+ had no appreciable change on EPS levels during the time frames. In the presence of Ag^+, 10 mM Ag^+ exposure increased toxicity slightly from 19.2 mg/L to 18.7 mg/L. However, the presence of 10 mM AgNPs and 10 mM Ag^+ had the greatest drying impact on EPS levels wherein EPS dropped from 8.5 mg/L to 7.9 mg/L indicating that AgNPs and Ag^+ could result in an increased inhibition of EPS levels from organisms possibly due to increased toxicity level and overload of cellular defenses from the total combination treatment (table 12).

Table 11. EPS Production in P. chrysosporium Treated with 2,4-DCP

2,4-DCP Concentration (mg/L)	EPS at 24 h (mg/L)	EPS at 48 h (mg/L)	Change Over Time
0 (Control)	14.9	-	-
25	22.4	~22.4	No significant change
50	20.0	~20.0	No significant change
100	37.9	27.5	Decreased (by ~10.4 mg/L)

1. Stimulated EPS production: *P. chrysosporium* increased EPS secretion under 2,4-DCP stress (peaking at 37.9 mg/L at 100 mg/L, 24 h), suggesting a stress response.
2. Concentration-dependent dynamics: EPS levels remained stable at lower doses (25–50 mg/L) but dropped by ~27% at 100 mg/L by 48 h, indicating potential toxicity or metabolic adaptation.
3. Control baseline: Unstressed cultures produced 14.9 mg/L EPS, confirming 2,4-DCP's role in modulating fungal biofilm matrices.

Table 12. EPS Production Under AgNP and Ag^+ Exposure

Condition	EPS at 24 h (mg/L)	EPS at 48 h (mg/L)	Change Over Time
10 mM AgNPs	11.5	11.0	Slight decrease
1 mM Ag^+	14.9	~14.9	No change
10 mM Ag^+ alone	19.2	18.7	Slight decrease
10 mM AgNPs + 10 mM Ag^+	8.5	7.9	Decrease

Ag^+ ions stimulate EPS more than AgNPs: At 10 mM, Ag^+ alone produced 19.2 mg/L EPS (24 h), while AgNPs yielded only 11.5 mg/L, suggesting ionic silver's stronger influence on EPS modulation.

Combined treatment suppresses EPS most effectively: AgNPs + Ag^+ caused the lowest EPS levels (8.5 → 7.9 mg/L), highlighting synergistic antibiofilm action.

Time-dependent decline: All AgNP/Ag^+ conditions showed gradual EPS reduction, implying sustained disruption of biofilm matrices.

Authors' Contributions

All authors equally contributed to this study.

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Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

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