Hindawi
Journal of Nanomaterials
Volume 2019, Article ID 7630316, 11 pages
https://doi.org/10.1155/2019/7630316

Review Article

Mechanisms of Resistance to Silver Nanoparticles in Endodontic Bacteria: A Literature Review

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Received 23 August 2018; Revised 12 November 2018; Accepted 29 November 2018; Published 20 January 2019

Academic Editor: Angelo Taglietti

In recent years, the use and research in nanomaterials have increased considerably. In dentistry, nanomaterials have been investigated in all their specialties like dental prosthesis, implantology, dental operative, periodontics, and endodontics. The nanomaterials are investigated in the areas of dentistry due to their application in the improvement of the physical and chemical properties of conventional materials, as well as the use of the antimicrobial activity of nanomaterials such as silver nanoparticles. Recently, silver nanoparticles (AgNPs) have been studied for their use as an endodontic irrigator due to their high antimicrobial activity. But little is known about the possible mechanisms of the adaptation to AgNPs by endodontic bacteria. These mechanisms may be intrinsic (such as efflux pumps, downregulation of porins, and chromosomal resistance genes) or extrinsic (such as point and adaptive mutations and plasmids with resistance genes) adaptation systems. In addition to this, it has been reported that coselection or coregulation of metal resistance mechanisms, as in the case of nanoparticles, is accompanied by increased resistance to various antibiotics. For these reasons, the objective of this article is to do a review of the literature on the possible mechanisms used by endodontic bacteria to generate resistance to silver nanoparticles and the possible side effects of these mechanisms.

1. Introduction

With the emergence of nanotechnology, silver nanoparticles (AgNPs) have been widely used in dentistry, mainly because of their antibacterial properties [1]. They are used in restorative dentistry through their incorporation in composite resins [2] and adhesive systems [3] in order to enhance their mechanical properties and prevent or diminish biofilm accumulation [4]. Silver nanoparticles are studied in dental prostheses where they are incorporated into polymers used as tissue conditioners and as denture bases to prevent the emergence of denture stomatitis. AgNPs are used in implantology to prevent biofilm formation over the implant surface. In endodontics, AgNPs have been incorporated into different materials (root canal sealer, cements, and gutta-percha) to prevent the recolonization of bacteria and have been studied as irrigating solutions and intracanal medication against bacterial biofilms [5]. This is due to the advantages that AgNPs offer in comparison to sodium hypochlorite (NaOCl). AgNPs maintain their antibacterial efficacy in the presence of dentin [6], and they are used as an alternative to root canal irrigation owing to their biocompatibility, especially in lower concentrations [7]. In addition, studies report that bacteria are not capable of developing resistance to AgNPs compared with antibiotics [8, 9]. Although not all mechanisms are well known, AgNPs can interact simultaneously with multiple targets in the microbial cell, like the cell membrane of both gram-positive and gram-negative bacteria [10, 11], enzymes, proteins [12], lipids [13], DNA, and plasmids [14], making it difficult for bacteria to generate resistance. These mechanisms have already been extensively reviewed [15, 16].

The mechanisms of resistance to silver nanoparticles have not been well studied, but there are reports of silver-resistant bacteria isolated from clinical and nonclinical

environments. The first clinical bacteria with silver resistance described was *Salmonella typhimurium* [17]. The mechanisms of resistance may be intrinsic and extrinsic. The intrinsic resistance mechanisms can include outer membrane permeability, multidrug resistance (MDR) efflux pumps [18], downregulation of genes [19], and chromosomal resistance genes [20]. The extrinsic mechanisms include point mutations, adaptive mutations, and plasmids with resistance genes. The objective of the present article was to realize a literature review focused on the mechanisms used by endodontic bacteria to generate resistance to silver nanoparticles. We hope this work can help and inspire further studies in the use of silver nanoparticles in the endodontic applications.

Electronic database searches from Scopus, PubMed, and Web of Science were performed up to and including October 2018. The exact search strategy used for retrieving the articles was as follows:

(1) "bacterial resistance" AND "silver nanoparticles"

(2) "biofilm resistance" AND "silver nanoparticles"

A secondary search was then conducted using the references or concepts mentioned in the selected articles in order to obtain more information.

2. Envelope Stress Response

Gram-negative bacteria have a structured envelope in a way that prevents the penetration of AgNPs. The outer cell envelope is composed of a bilayer formed by lipopolysaccharide (LPS), phospholipids, and proteins (like porins) [21]. The gram-positive bacterial wall is composed of teichoic acids (TAs) and peptidoglycan that offers less resistance to the passage of certain substances [22]. Despite the aforementioned differences, in general, both bacteria have a negative charge in their envelope [23]. The envelope's negative charge of gram-positive bacteria is due to the presence of TAs. TAs are polyanionic, phosphate-rich linear polymers [24]; the phosphate group is one of the three main groups, together with the carboxyl and amino groups, responsible for the negative charge of the bacterial cell membrane [25]. The negative charge of gram-negative bacteria is given by the presence of lipopolysaccharide, more specifically by the lipid A. The structure of lipid A is phosphorylated with hydrophilic carbohydrates (core oligosaccharide). This type of carbohydrates is the main component that gives a negative charge to the bacteria's envelope [26].

The surface charge of the AgNPs influences their antimicrobial action and selectivity over some species of bacteria. Abbaszadegan et al. reported that the antibacterial activity of silver nanoparticles depends on the electrical charge of their surfaces. They tested the antimicrobial activity of silver nanoparticles with three different electric charges (negative, positive, and neutral) on gram-positive (*Streptococcus mutans*, *Streptococcus pyogenes*, and *Staphylococcus aureus*) and gram-negative (*Proteus vulgaris* and *Escherichia coli*) species. They showed that nanoparticles with a positive charge had the highest antimicrobial activity followed by the ones with a neutral charge and a negative charge, respectively. The antibacterial effect seemed to be independent of the size of the nanoparticles, giving greater importance to the surface charge. But *Proteus vulgaris* has the major resistance to the three types of nanoparticles assessed, and the maximum concentration of the nanoparticles with a positive charge had to be used to achieve minimal inhibitory concentrations on *Proteus vulgaris* [27]. Mandal et al. found a greater internalization of the AgNPs in gram-positive bacteria because they presented a less negative charge on its surface (−15 mV) than gram-negative bacteria (−26 mV). Gram-negative bacteria tend to repel silver nanoparticles because their negative charge has a closer value to the one reported for the silver nanoparticles (−32.2 mV) when measured by dynamic light scattering (DLS). These differences in the electrical charges influenced the antimicrobial activity of the silver nanoparticles. In a study performed by Mandal et al., a higher antimicrobial activity of AgNPs was observed in *Enterococcus faecalis* cultures (MIC = 5 μg/mL) due to the difference in zeta potential values between *E. faecalis* and AgNPs, while the antimicrobial activity on the *P. vulgaris* cultures was lower (MIC = 10 μg/mL) due to a negative repulsion between them and AgNPs [28] (Figure 1).

The existence of bacteria with resistance to AgNPs, along with different types of electric charges in their surfaces, can be explained in part by the presence of envelope stress response mechanisms. The functions of these mechanisms are detected in response to harmful stimuli that may affect the bacterial cell envelope [29]. The envelope stress response is formed by three essential mechanisms present in gram-positive and gram-negative bacteria. These defense mechanisms can be activated by a wide variety of stimuli such as oxidative stress, osmotic stress, and alkaline pH. The first system is formed by a family of alternative sigma factors called extracytoplasmic function sigma factors. These sigma factors regulate the expression of genes with known and unknown functions [30]. The most well-known function of these factors is in the biogenesis of lipopolysaccharides. This function is related to the generation of resistance to nanoparticles with different charges on their surface; in this context, the aforementioned factors participate in the mechanisms for the incorporation of D-alanine into the polyanionic TAs, which results in the reduction of the negative net charge of the cell wall of gram-positive bacteria. This mechanism also participates in the generation of resistance to cationic antibiotics and cationic antimicrobial peptides (CAMPs). Therefore, the bacteria are able to regulate the charge of its envelope to lower its affinity to AgNPs [31]. A similar mechanism occurs in gram-negative bacteria and lipid A present in its cell wall [32].

The second component of the envelope stress response systems is named two-component signal transduction (TCS) systems. As the name implies, these systems are formed by two components, a sensor kinase (a transmembrane protein) and a response regulator (a cytoplasmic protein). The most studied of this type of systems is the conjugative pilus expression (Cpx) system. This system regulates the expression of a wide variety of genes and proteins, for example, the expression of virulence factors like

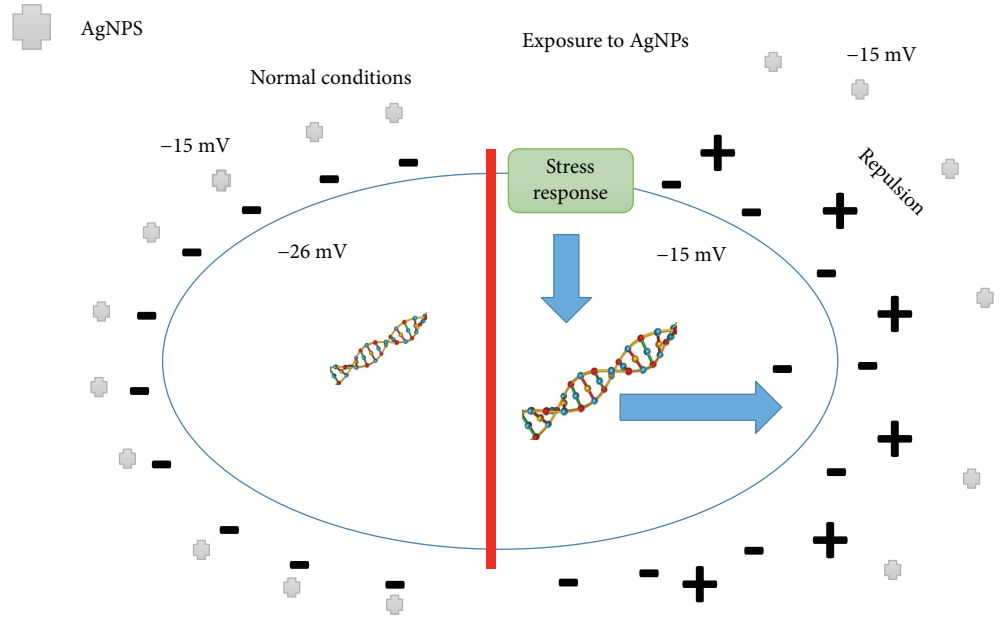

Figure 1: The difference in zeta potential values between bacteria (−26 mV) and AgNPs (−15 mV) exerts attraction and greater contact of AgNPs and bacteria. The envelope stress response is activated in the presence of AgNPs and incorporates positive charges into the bacterial cell wall. The incorporation of positive charges allows the bacteria to equalize the electrical charge with the surface of the AgNPs causing its repulsion.

pili/fimbriae factors. Recently, it was found that the overexpression of proteins constituting the surface appendages of bacteria like flagellin by *E. coli* served as an extracellular matrix that upon contact with AgNPs caused its agglomeration and inactivation [33].

LiaFSR is a system in charge of the protection mechanisms against antibiotics in gram-positive bacteria [34]. The LiaFSR system is homologous to the TCS system, and its operon is formed by at least three genes: the first gene, *liaS*, encodes for a sensor with double function, the second gene, *liaR*, encodes for a response regulator, and the last gene, *liaF*, encodes for a transmembrane domain protein. The function of this gene is to negatively regulate the transcriptional effects of *LiaR*. This system is highly conserved in a certain group of bacteria (low G+C bacteria) integrated by relevant human oral pathogens including *Enterococcus faecalis*. LiaFSR also controls the regulation of promoters (*PliaI*) involved in the formation of dormant endospores [35] and biofilm and is a master regulator of the envelope stress response mechanisms [34].

BaeSR is also a TCS system that participates in the overexpression of multidrug efflux pumps from the RND (resistance, nodulation, and cell division) family related to metal and antibiotic resistance [36]. In addition, TCS systems can stimulate other defense pathways in microorganisms. Likewise, other systems can stimulate the activation of TCS systems in the same way [37].

The third component of the envelope stress response systems is the phage shock protein (PSP) response, although the majority of its function are unknown and information is scarce.

2.1. Clinical Significance. Endodontic infections originated from a group of microorganisms with a great diversity of bacterial species. These bacteria are usually embedded in a matrix of exopolymers commonly called biofilm. As mentioned above, all types of bacteria may have different mechanisms of resistance to silver nanoparticles and further characteristics, such as size and charge. Therefore, in their clinical use, it is difficult to establish parameters, so that the antimicrobial activity of the AgNPs exerts a uniform effect on the wide range of microorganisms present in the root canal system of teeth that need endodontic treatment. The biofilm formation and the different mechanisms of resistance to silver nanoparticles could favor the survival of certain species of bacteria that could adapt to the silver nanoparticles, transmit the mechanisms, and give rise to resistance even against antibiotics.

3. Bacterial Persisters

Persistent bacteria are a bacterial subpopulation that has an altered phenotype that allows it to escape the effect of antibiotics, disinfectants, and various harmful stimuli. Persistent bacteria involve about less than 1% of the cells in a bacterial population. They use different mechanisms to survive in comparison to the well-known concept of resistance resulting from genetic mutations or horizontal gene transfer. The first persistent bacterium that was described is *Staphylococcus aureus*, and it was reported by Joseph Bigger in 1944 [38]. The existence of persistent cells has a great clinical significance because it is linked with the development of chronic bacterial infections [39]. In endodontics, persistent bacteria are related to the development of secondary and recurrent endodontic infections [40]. When silver nanoparticles are used as irrigating solutions or as an additive into endodontic materials, the bacteria could be exposed to silver

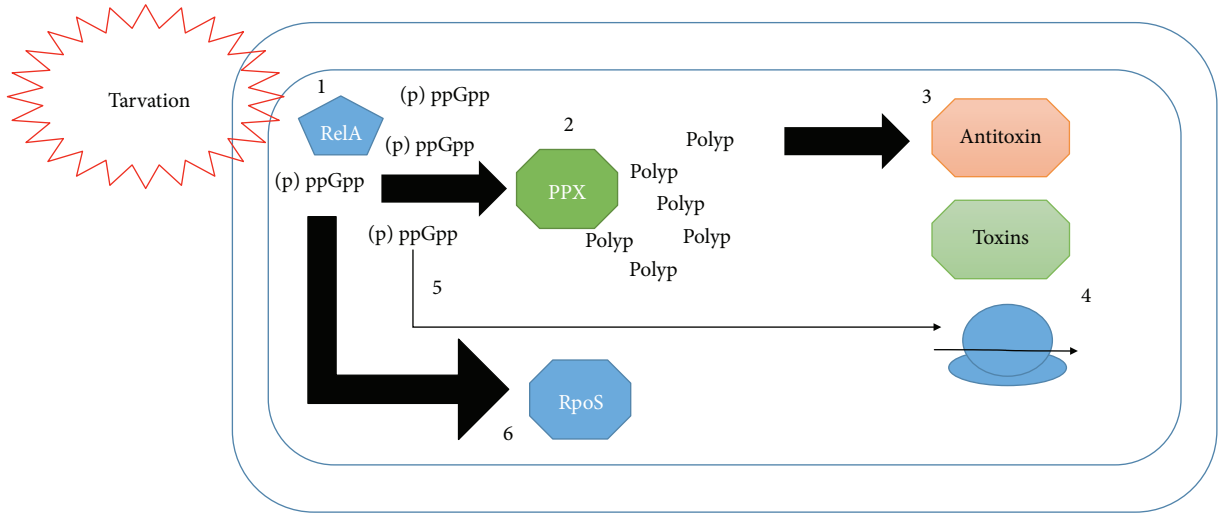

Figure 2: Appearance of a persistence phenotype. (1) Activation of RelA (by starvation and several stimuli) promotes the accumulation of ppGpp. (2) The ppGppppGpp inhibits the exopolyphosphatase (PPX), the enzyme that degrades polyP, promoting the accumulation of polyP. (3) PolyP causes the degradation of antitoxins. (4) Free and activated toxins induce persistence. (5) The accumulation of ppGpp interferes with mRNAs and induces the appearance of a persistence phenotype. (6) Finally, ppGpp also stimulates the accumulation of RpoS, a master regulator of the general stress response.

concentrations lower than the minimum bactericidal concentration (MIC), but this concentration of silver could be enough to cause oxidative stress. Exposure to low concentrations of AgNPs causes oxidative stress in bacteria and stimulates the presence of persistent bacteria [41]. Studies have shown that *Enterococcus faecalis* is the bacteria with the highest prevalence in root canal-treated teeth and also presents a high rate of persistent phenotypes [42]. For example, when *E. faecalis* is found in environments with little availability of nutrients, it can adopt a viable but noncultivable state [43], a survival mechanism used by oral bacteria in multispecies oral biofilm exposed to adverse conditions [44]. *E. faecalis* can also adopt a dormant state (up to 12 months) when there are not enough nutrients and leave that state when there are nutrients available [45], especially in coexistence with other species like *Streptococcus gordonii* or *Candida* [46].

The pathways by which bacteria are considered to develop "persistent" phenotypes are through two types of mechanisms: mechanisms of active and passive defense. The passive defense mechanisms consist of molecular mechanisms of dormancy like toxin-antitoxin modules, guanosine tetraphosphate (ppGpp), guanosine pentaphosphate (pppGpp), and indole, while the active defense mechanisms studied for the development of persistent bacteria are efflux pumps [47] (Figure 2). Cell dormancy is considered the main mechanism to generate persistent bacteria. It can be defined as a state where the antibiotics still bind to their targets, but the drugs cannot exert their lethal effects due to the inactivation of downstream pathways [48]. The mechanism that is most studied is the toxin-antitoxin modules, which are divided into six main types that are classified according to the nature of the antitoxin. In types I and III, the antitoxin is a noncoding ribonucleic acid (RNA), whereas in types II, IV, V, and VI, the antitoxin is a protein. In general, the system works when the bacteria synthesize a toxin that acts like an RNase that degrades the messenger RNA (mRNA) resulting in the induction of persistent bacteria. The toxin produced is neutralized by an antitoxin also encoded by the DNA (deoxyribonucleic acid) of the bacteria [49]. On the other hand, (p)ppGpp is a nucleotide second messenger that induces large-scale transcriptional repression of genes involved in amino acid biosynthesis, stress response, nutrient acquisition, translation factors, and the activities of enzymes involved in GTP (guanosine triphosphate) biosynthesis, which are usually essential for the rapid growth of bacteria [50]. Indole is an intercellular signaling molecule that can trigger protective responses and create a persistent subpopulation of bacteria [51].

The active defense mechanisms of the persistent bacteria are the efflux pumps [52]. Pu et al. proved that the accumulation levels of antibiotics were considerably lower inside persistent bacteria employing fluorescent antibiotic and single-cell microscopy. The subsequent transcriptome analysis of the persistent cells revealed that a group of efflux pump genes was expressed at significantly higher levels in persistent bacteria, with higher efflux rates for antibiotics compared with the rates of normal cells [53]. These efflux pumps participate in the generation of resistance to AgNPs through the efflux of silver ions [54]. The release of silver ions is one of the mechanisms by which silver nanoparticles with sizes of 20–80 nm exert their antimicrobial activity [55].

3.1. Clinical Significance. Besides the aforementioned mechanisms, endodontic bacteria can use several strategies to resist the chemomechanical preparation during root canal treatment. The complicated anatomy of the root canals facilitates the adhesion of bacteria and the formation of biofilms, and this stimulates the appearance of the different resistance phenotypes mentioned above [56]. Bacteria located in anatomy irregularities like ramifications and isthmi can also escape the effects of the chemomechanical preparation due to an inadequate disinfection [57]. Bacteria can also penetrate

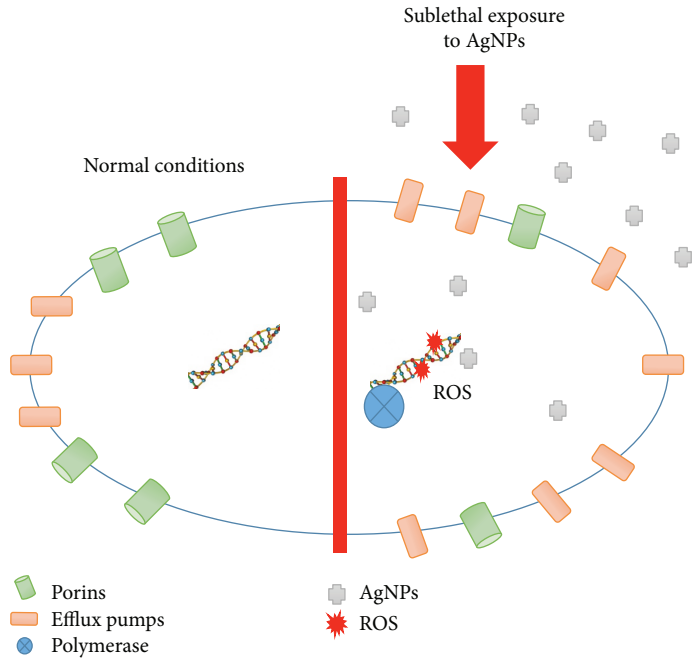

Figure 3: Nonlethal doses of reactive oxygen species (ROS) produced by AgNPs induce the expression of a special type of polymerase with the ability to repair DNA damage and the high degree of mutations induced by ROS [65]. Mutations produced by the increasing ROS can upregulate efflux pump genes and downregulate porins causing a resistant phenotype in bacteria.

deeply into the dentinal tubules avoiding a direct contact with the AgNPs and only being exposed to nonbactericidal concentrations. An example of this is *Enterococcus faecalis* that can penetrate into the dentinal tubules up to 1500 μm [58, 59], while some studies report that silver nanoparticle can only penetrate up to 1000 μm into the dentinal tubules [60].

4. Mutations

Nonlethal concentrations of AgNPs can increase the mutation rate of bacteria due to oxidative damage [61], DNA damage [62], and general stress responses [63]. Bacteria in contact with nonlethal doses of AgNPs can generate resistant phenotypes as a result of the increase of mutations (becoming transient hypermutators) by the production of nonlethal doses of reactive oxygen species (ROS). Small-size (≤10 nm) AgNPs can cross the wall and bacterial membrane and increase the production of ROS by inhibiting respiratory chain enzymes and promoting their accumulation inside bacteria [64]. High levels of ROS activate the SOS response that induces the expression of a special type of polymerase with the ability to repair DNA damage and the high degree of mutations induced by ROS [65]. Hence, the mechanisms by which the AgNPs have antimicrobial action can favor the appearance of bacteria with resistant phenotypes when exposed to nonlethal doses.

Experimental evolution was used to test the development of resistance to silver nanoparticles by a naïve *E. coli* strain. When *E. coli* was exposed to different concentrations of citrate-coated silver nanoparticles (10 nm), a greater formation of colonies was observed than that of the control group; this fact can be associated with the bacterial adaptation to bacteriostatic and bactericidal concentrations. In the genome analysis, the authors reported that bacteria have developed two types of mutations, single-nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (indels). Three SNPs were detected in the experimental group of bacteria. The first was in the *cusS* gene, which is responsible for sensing the concentration of copper ions and activating the expression of the *CusCFBA* efflux system in the *E. coli* genome. This system is homologous with *silCFBA* that is found in the pMG101 plasmid of *Salmonella typhimurium* and is responsible for the efflux of silver ions [66]. The second mutation was in the *purL* gene, which participates in the purine nucleotide biosynthesis [67]. The third was in the RNA polymerase beta subunit, *rpoB*, which might cause a change in the expression of a large number of genes [68]. On the other hand, of all the indels detected in this study, three seem to be particularly important. These three indels were found in the outer membrane protein R (OmpR) that is a member of a subfamily of response regulators and a DNA-binding protein also involved in the expression of a large variety of genes, like the genes that express the porins, ompF, and ompC [69] (Figure 3).

4.1. Clinical Significance. The bacteria present in endodontic infections are anaerobic; therefore, due to the lack of oxygen, ROS production by AgNPs would be minimal. Low levels of ROS would stimulate the presence of resistance due to oxidative stress and increased mutation rates in bacteria. Mutation of efflux pumps can undergo regulation which has been shown to elevate the minimum inhibitory concentrations (MICs) from 2 to 8 times, and the spectrum of resistance may include a wide range of antibiotics. In addition, bacteria can undergo mutations that lead to downregulation of

different proteins (like porins) which also contributes to the development of resistance by preventing the access routes of the AgNPs [70]. As mentioned before, bacteria in dormancy state maintain active efflux systems, so they can quickly expel bactericidal compounds if they penetrate their defenses [53]. For example, it was reported that silver-resistant bacteria (*Escherichia coli*) had an augmented active efflux of silver ions and had reduced permeability due to a lower number of porins (like OmpF or OmpC) present in their outer membrane [19]. These efflux systems also participate in the development of resistance to intracanal medicaments and irrigating solutions in endodontics [71, 72], although some reports are contradictory. Evans et al. and Brändle et al. concluded that survival of *E. faecalis* exposed to calcium hydroxide seems to be given by an efflux pump because they observed a large decrease in *E. faecalis* survival when an efflux pump inhibitor (EPI) was used [73, 74]. Upadya et al. reported that the use of an EPI improved the antibiofilm efficacy of light-activated disinfection (LAD), chitosan nanoparticles, and $Ca(OH)_2$, showing the participation of the efflux pump systems in antimicrobial resistance [75].

5. Plasmids

Plasmids are extrachromosomal genetic elements that are found inside the prokaryotic cells, where they replicate independently from the chromosome. Plasmids can enter bacterial cells through both active and passive mechanisms. These characteristics make them important agents involved in the lateral transfer of resistant genes to antimicrobials such as silver nanoparticles.

The mercury resistance plasmid was the first of 12 metal resistance plasmids (including those for silver, copper, nickel, and zinc) to be described. The plasmid encodes for a transport system that binds to mercury ions (Hg^+) to transport them to the cytoplasm where they are inactivated by a specific reductase enzyme. It is known that the oral hygiene habits, mastication, and polishing can increase the intraoral mercury (Hg) vapor from dental amalgams [76], which contains 30-50% metallic mercury. Some studies report that mercury levels in plaque from amalgam surfaces are significantly higher than those from plaque on enamel surfaces [77]. And low and high copper amalgams used in recent years also have high mercury release rates. It was reported that mercury released from dental amalgams stimulates the appearance of resistant bacteria (*streptococci* and *enterococci*) in oral and intestinal floras of primates [78]. Several studies indicate that the presence of plasmids that generate resistance to mercury and antibiotics is high in various oral bacteria. Pike et al. reported a high prevalence of mercury-resistant bacteria in children with and without amalgams. In addition, there was a slight increase in resistance to antibiotics in bacteria with mercury resistance, but such increase is not significant [79].

Dental amalgams are also a source of exposure to silver since they contain approximately 35% of silver [80]. Bacterial resistance to silver is encoded by genes that are found both in plasmids and in the bacterial chromosome [81]. The first plasmid to encode bacterial Ag^+ resistance was isolated from *Salmonella typhimurium* in the Massachusetts General Hospital in 1975. The plasmid was named pMG101 and confers resistance to both silver and mercury, as well as a wide variety of antibiotics (amoxicillin, ampicillin, and tetracycline) [17].

This plasmid contains a 14.2 kb region (*sil* operon) with nine ORFs (open reading frames) arranged in three transcriptional units (*silCFBAGP*, *silRS*, and *silE*) expressed from a different promoter [81]. These transcriptional subunits are collectively designated as the *sil* operon. The first transcriptional unit is composed of *silCBA*, which is immediately upstream of *silRS*, and is transcribed divergently from it. This transcription unit encodes a protein complex consisting of *SilA* (a proton/cation antiporter) and *SilB* and *SilC* (two structural proteins bound to the inner and outer membrane) [82]. These three components form an efflux pump of the type cation/proton antiporter that belongs to the RND (resistance, nodulation, and cell division) transporter family [83]. *SilG* and *silF* are periplasmic silver chaperones. *SilF* is homologous to *CusF* and participates in the transportation of silver ions to *SilCBA* [84]. Finally, *silP* is believed to be an efflux pump belonging to the ATPases (P-type) that participate in the heavy metal resistance [85].

In the second transcription unit, there are a couple of genes named *silRS*. *SilS* encodes for a protein that is responsible for sensing the levels of silver ions in the environment, while *silR* encodes for a protein responsible for initiating the transcription of the other genes in response to the presence of silver ions [86]. The last transcriptional unit is composed of *silE* that encodes for a periplasmic metal-binding protein responsible for trapping silver ions present in the environment [82]. Finally, there is a 96-codon open reading frame of unassigned function between *silC* and *silB*, and there is a 105-codon open reading frame of unassigned function between *silA* and *silP*. The silver resistance by plasmids can be improved by endogenous (chromosomic) operons named *cus* and *cue* operons [87] and cooper resistance plasmids like *pco* and *cop* [88]. The *cus* and *sil* systems are closely related homologues (67-80%). The *cus* system is formed by *CusRS*, which encodes for two proteins whose function is to regulate the activation of the *cus* operon (*cusCFBA*). *CusF*, like *silF* and *silG*, is a chaperone protein that is responsible for trapping metal ions (copper ions). *CusCBA* encodes for a silver/-copper efflux pump formed by three proteins belonging to the RND family. The *cus* system is induced under higher external levels of copper and can be coregulated or coexpressed with the *sil* operon [89]. The RND family that encodes for multidrug efflux systems has an important role in the development of resistance to a wide range of antimicrobials in gram-negative bacteria, and they have been recently cataloged as part of the bacterial stress responses [90].

5.1. Clinical Significance. The presence of these mobile genetic elements has not been studied in bacteria isolated from endodontic infections. There is only one report of the presence of these plasmids and the silver-resistant genes in oral bacteria [91]. Hence, more studies of the presence of these plasmids and genes in the oral cavity are necessary to prevent their clinical repercussions. The presence of these plasmids and genes have been reported in *Enterococcus*

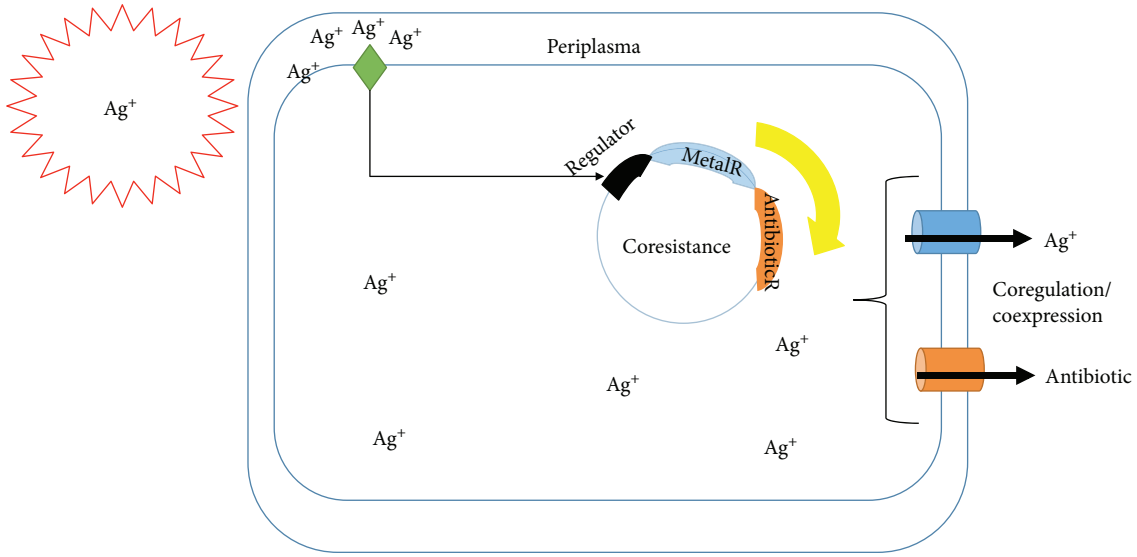

FIGURE 4: Mechanisms of cross-resistance to metal and antibiotic resistance. One plasmid confers resistance to both antibiotic and metal (*sil* operon) (coregulation/coexpression) when the expression of both elements of resistance is controlled by a common regulator within one single plasmid.

genus, *E. cloacae*, and *K. pneumoniae* isolated from human diabetic foot ulcers and wounds from a tertiary care facility [92, 93]. These bacteria are also found in recurrent endodontic infections [94, 95]. Endodontic bacteria like *E. faecalis* have virulence factors like sex pheromones that can increase by several folds the transfer frequency of plasmids facilitating the transfer of phenotypes resistant to silver nanoparticles [96, 97].

6. Coselection and Coregulation of Antibiotic and Metal Resistance

Coresistance occurs when the genes for different resistant phenotypes are located on the same mobile genetic element. These elements may include plasmids, transposons, or integrons commonly found in bacterial genomes or extrachromosomal elements [98]. The plasmids, as mentioned before, are extrachromosomal genetic elements that can code for various proteins that give the bacteria extra features such as resistance to metals. Integrons and transposons are gene acquisition systems that allow obtaining, propagating, and expressing genetic elements that contain genes resistant to a wide range of antimicrobials, especially in gram-negative bacteria. The coresistance to antibiotics and metals in bacteria has been studied for several decades, more precisely since 1974 when multiple antibiotic and metal-resistant strains of *E. coli* were identified in sediments [99]. In odontology, the percentages of mercury and silver released from dental amalgams have been associated with the coselection of antibiotic and metal-resistant bacteria [78]. In addition, independent mechanisms of resistance to metal or antibiotics can be coregulated allowing the orchestration of a mixed response to different harmful stimuli. For example, the *mdtABC* operon that encodes a RND efflux system can be upregulated in response to high levels of zinc in some *E. coli* strains, and this upregulation can cause the development of antibiotic resistance [100] (Figure 4).

6.1. Clinical Significance. The increased use of silver nanoparticles in various dental materials might stimulate the appearance of coresistance and coregulation to metals and antibiotics. Therefore, bacteria would develop resistance to antibiotics when exposed to nonbactericidal concentrations of AgNPs. This type of costimulation has been reported with the use of dental amalgam [101]. This would generate superinfections that could compromise the systemic health of the patients [102].

7. Biofilm

Biofilms are considered to be the predominant growth phenotype of bacteria in endodontic infections and the major cause of both primary and secondary root canal infections [103]. Bacteria inside a biofilm have resistant phenotypes due to the transfer of mobile genetic elements between them and the expression of resistant mechanisms [104]. For example, after exposure to nonlethal concentration of polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs) with 10 nm size, *Pseudomonas aeruginosa* PAO1 presented an increase in biofilm development and upregulation of antibiotic resistance genes (ARGs), lipopolysaccharide biosynthesis, quorum sensing, and increased extracellular polymeric substances (like sugar and proteins). The results of the aforementioned study show that the biofilms promote the expression of different resistance mechanisms. And the expression of different resistance mechanisms results in a more effective response against the antimicrobial activity of the AgNPs (Figure 5).

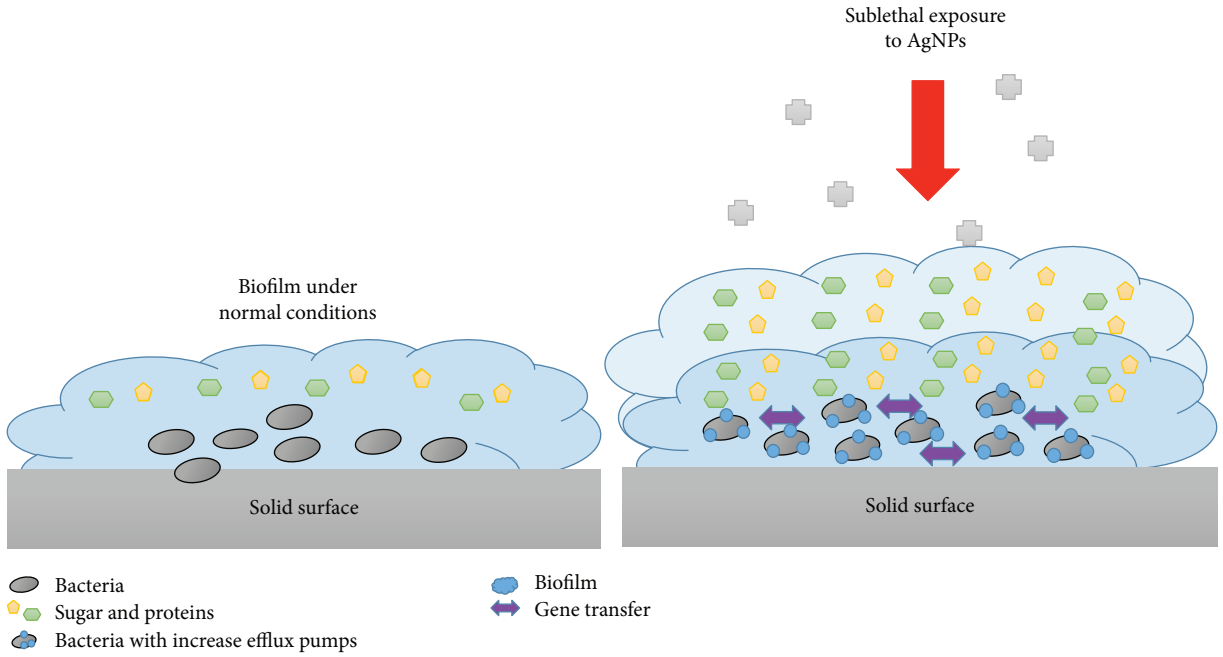

Figure 5: Sublethal exposure to AgNPs enhanced biofilm development, upregulated lipopolysaccharide biosynthesis, gene transfer, and efflux pump genes, and increased the sugar and protein contents of the biofilm.

8. Conclusion

Clearly, there are multiple resistance and persistence mechanisms that can act together to develop AgNP resistance in bacteria. Hence, the clinical use of silver nanoparticles could stimulate the appearance of resistance in bacteria in a short period of time. We consider that it is important to study all the aforementioned mechanisms in different endodontic bacteria to understand the resistance to AgNPs and to prevent side effects such as increased resistance to antibiotics. Perhaps, guidelines should be developed to regulate the interaction of AgNPs with organisms and the environment.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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