Review Article

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# Combatting persisted and biofilm antimicrobial resistant bacterial by using nanoparticles

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**Abstract:** Some bacteria can withstand the existence of an antibiotic without undergoing any genetic changes. They are neither cysts nor spores and are one of the causes of disease recurrence, accounting for about 1% of the biofilm. There are numerous approaches to eradication and combating biofilm-forming organisms. Nanotechnology is one of them, and it has shown promising results against persister cells. In the review, we go over the persister cell and biofilm in extensive detail. This includes the biofilm formation cycle, antibiotic resistance, and treatment with various nanoparticles. Furthermore, the gene-level mechanism of persister cell formation and its therapeutic interventions with nanoparticles were discussed.

**Keywords:** biofilm; nanoparticle; persister.

## 1 General consideration about bio-films and persisted cells

Bacteria, the simplest organism, is the most ancient and one of the key components required by the biome, as it is responsible for the majority of work at lower levels, such as the micro level [1]. They adapted to every environment on the earth's surface, from high-temperature volcanic regions to deep freezing environments, and they used plants and animals as a means of survival [2]. These bacteria existed in the form of a biofilm or a cell (planktonic), according to Refs. [1, 3–6]. Biofilms differ from planktonic cells in composition because they live in nature, reproduce, and exist in colonies in three-layered structures such as complex three-dimensional (3D) shapes [1, 4, 5]. They are made up of a single or multiple bacterial species. Depending on the species and environment, it responds differently and exhibits similar properties [1, 3–5]. The bio-films are held together by a sticky mass known as extracellular polymeric substance (EPS), which is a polymer excreted by microbes. They can interact with biological and non-biological materials in both specific and non-specific ways [3, 5]. Bio-film cells produce a sticky mass of EPS that is strongly held together by these strands, giving them a complex, three-dimensional structure [4]. All biofilms vary in size and shape depending on environmental conditions, nutrient availability, and growth status [1, 3]. Bacterial cells are protected from technical accents due to the viscoelastic nature of biofilms [1, 5]. The biofilm growth cycle begins with the formation of bacterial cells and ends with the formation of new sister cells (Figure 1A) [6]. Throughout the cycle, biofilm serves as a mediator of cell signals as well as a medium for metabolic activities [6].

Persister cells are resistant to antimicrobial treatments due to a decrease in metabolic activity, which is dependent on corrupting active biochemical pathways. As a result, some molecules responsible for the killing of bacterial persister cells should function properly. cisplatin and the DNA-crosslinker mitomycin C are included in the antibacterial precursor compound. Persisters' function is to withstand stress caused by inactive metabolic activity [7–10] and without genetics [11]. The nongrowing cell of *Staphylococcus aureus* that tolerates penicillin stress is a practical example of presister [7, 8]. As presister cells used to exist in a small subpopulation, they normally resist when mutagenic changes occur that allow the antibiotic to be used, whereas tolerance occurs when growth is reduced, making the entire population less susceptible to the antibiotic [12, 13]. Scientists tried to clear up the confusion regarding the literature of presisters [14–18]. They also tried to highlight the mistakes being made by the researchers by not waiting for the plateau in the graph where some viable cells retain and shoe the existence of presister cells [19]. It was reported that for selected bacteria such as enterophemorrhagic *Escherichia coli* and *E. coli*, the viable fraction is similar to that of their presister cells [16].

Persisters are made up of various components such as oxidative stress, nutrients, and antibiotics [16, 20]. The majority of cells are thought to be underfed [21], and in a persistence condition, archea and bacteria are thought to be in a resting stage [19]. Persistence used to exist in a

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limited capacity, but its response to the environment is highly regulated [22–26]. In biofilms and stationary-phase cultures, a small subpopulation (±1%) of stress-tolerant cells responds to the environment [27, 28].

# 2 Biofilm resistance formation and treatment using nanoparticles

## 2.1 Formation of bio-film formation its resistance effect

### 2.1.1 Cycle of bio-film showing its development

The biofilm development cycle begins with the formation of a 3D structure and the attachment of bacterial cells that exist freely on living or nonliving surfaces while keeping in mind the feasible environmental conditions and ends with the secretion of EPS in the 3D shape of biofilms [29]. The major component of biofilm is EPS, which accounts for approximately 90% of total biomass; biofilm is composed of lipids, protein nucleic acid, and carbohydrate polysaccharides [30]. The function of EPS is to keep biofilm bacteria together, as well as to allow cell-to-cell communication, gene transfer, and antibiotic resistance in bacteria. Furthermore, EPS provides minerals to bacteria, such as phosphorus, nitrogen, and carbon, in addition to the compounds [30]. When the biofilm progresses, the bacteria from the biofilm are excreted and dispersed from the colony, attempting to form new colonies in search of new and better nutrients [29]. Various plans and strategies were used at each stage of biofilm development. Mannosides, curlicides, and pilicides have been used as anti-adhesion

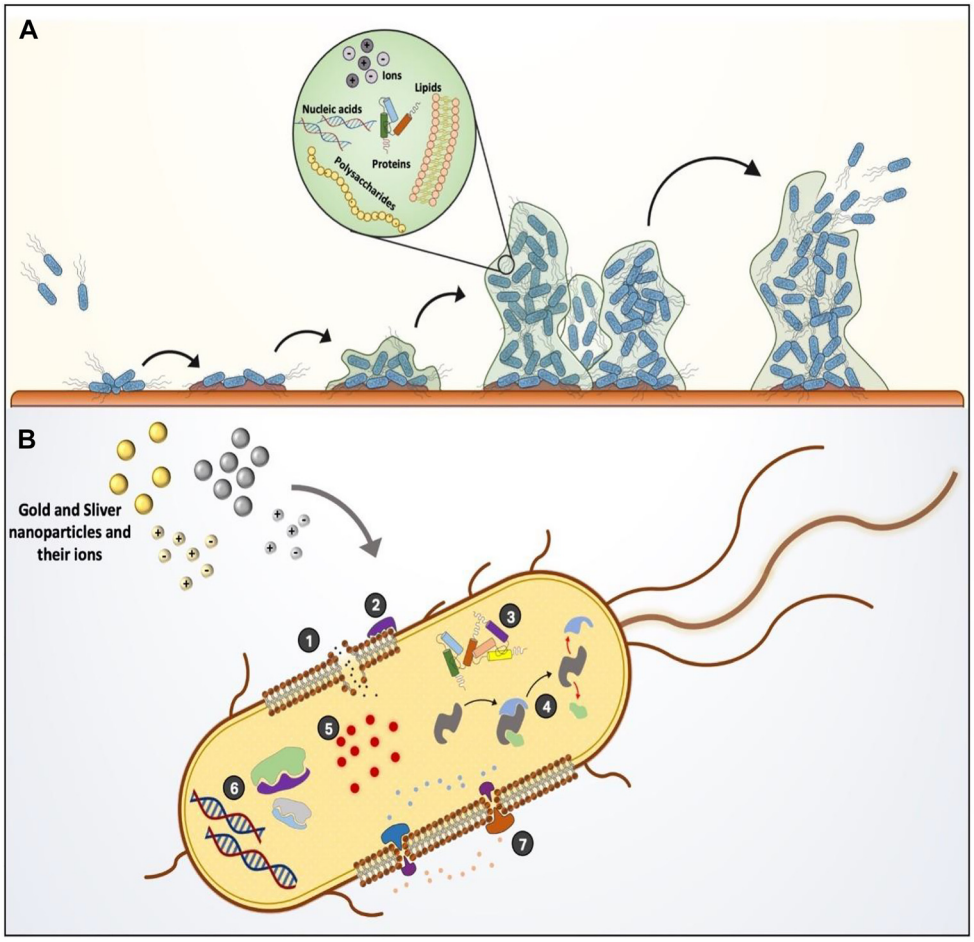


**Figure 1:** (**A**) This image depicts the formation of a bio-film and its attachment to biological and non-biological surfaces, as well as its adhesion, reproduction, and EPS secretion. Figure A also depicts the multilayer three-dimensional shape of a biofilm made up of major (nucleic acid, polysaccharides, protein) and minor (lipids, ions, water) components (**B**) The image depicts the effect of gold and silver nanoparticles and their ions on seven specific target sites within a bacterial cell. The molecular structure and bacterial cell are not to scale, but are represented arbitrarily for symbolic purposes.

agents, and some anti-biofilm polysaccharides can also be used to prevent bacteria from adhering to the surface [31–34]. The maturation of biofilms and the formation of micro-colonies can also be delayed by using silver NPs, lytic phage, and some enzymes such as EPS-degrading enzymes [35, 36]. Before the natural dispersal of signals occurs, the bio-film bacterial cells spread out in the surrounding medium to control signal dispersion [37].

## 3 Resistance of bio-film and antibiotic

It was found that two years after the introduction of penicillin, resistance to the antibiotic effect took two years [38]. Antibiotic resistance has proven to be a challenge for the healthcare system, posing a burden on the system as a result of the widespread use of antibiotics [39], whereas biofilm is crucial in combating the health crisis.

Understanding the biofilm antibiotic resistance mechanism is critical for treating biofilm-related diseases. Antibiotics work in a much mechanized way as they attack the bacterial cell and target the cell wall biosynthesis, protein synthesis, and DNA replication and repair processes. According to the literature, bacterial cells show resistance to these attacks through a variety of mechanisms, including enzymes that deactivate antibiotics, efflux pumps, and reprogramming of antibiotic targets [39]. The function of efflux pumps is to pump antibiotics out of bacterial cells via membranes in order to reduce antibiotic concentrations within the cell. The role of enzymes in antibiotic resistance is also very beneficial because it deactivates antibiotics through specific modifications within their components. Antibiotics' targeted sites are modified over time to avoid resemblance [38]. The antibiotic resistance of biofilm bacteria differs from that of planktonic bacteria, whereas the mechanisms of planktonic bacteria are easily understood. Understanding planktonic bacterial antibiotic resistance in comparison to biofilm bacteria is a difficult task. The aforementioned mechanism may exist in biofilms; these antibiotics work in a similar manner with various mechanisms for the specific mode of biofilm growth. While the correct mechanism is still being researched, scientists are working on several mechanisms to precisely explain the status of biofilm antibiotic resistance. Although some well-known mechanisms are mentioned in the literature, Stewart [40] is easily accessible. It was previously thought that the 3D structure of biofilm served as a physical barrier to prevent antibiotic diffusion within the body. However, recent research claims that antibiotic diffusion is unrelated to biofilm structure [41, 42]. Furthermore, other literature supports the hypothesis that once an antibiotic binds to the polysaccharides, proteins, and DNA present in the biofilm EPS, it becomes inactive on the targeted bacteria and loses its ability to kill them [43]. When bacterial cells are lysed, genetic components such as plasmids are released and remain in the media, enhancing the process of gene transfer among bacterial cells [33]. The plasmids contain antibiotic resistance genes, which are thought to be beneficial [44, 45] formalized paraphrase It was concluded that antibiotics such as beta-lactams are lysed by carbapenemases, which is the literature's conformation for gene transfer resistance mechanism. Meanwhile, the interior structure of biofilm reveals that because it is anaerobic in nature, its bacteria have access to a limited supply of oxygen and other nutrients, making antibiotics less effective against bacterial killing [46]. Penicillin begins to disrupt bacterial cell synthesis; if the biofilm is not synthesizing a cell wall, penicillin will be ineffective. It is claimed that within biofilm, bacterial cells are in various stages of growth, allowing some populations of bacteria to survive antibiotic attack. According to Persister theory, very few bacterial cell populations are resistant to antibiotic attack, and intervention results in the incomplete killing of bacterial cells [47, 48]. Another mechanism used to control genes is quorum sensing, which controls genes by sensing local cell density [49], and which has also actively participated in the formation of Refs. [50–53]. It is still unclear how the biofilm antibiotic is controlled by quorum sensing, but it has been reported that efflux pump genes use quorum sensing, as reported by Ref. [54]. The current literature also supports and points to a link between quorum sensing and antibiotic resistance [55]. There is very little literature mentioned here, demonstrating that it is a complex process (biofilm antibiotic resistance) and an alarming challenge.

## 4 Treatment of biofilms with nanoparticles

Two types of nanoparticles (NPs) should be used to combat bacterial infections. The first is made of lipids, polymers, and silica and serves as a drug delivery carrier to activate antibiotics, while the second is made of metallic NPs and serves as an antimicrobial agent. When used as a drug carrier, NPs protect antimicrobial agents from enzymes such as lactamases, function to prevent antimicrobial agents from sticking to EPS components such as DNA, and

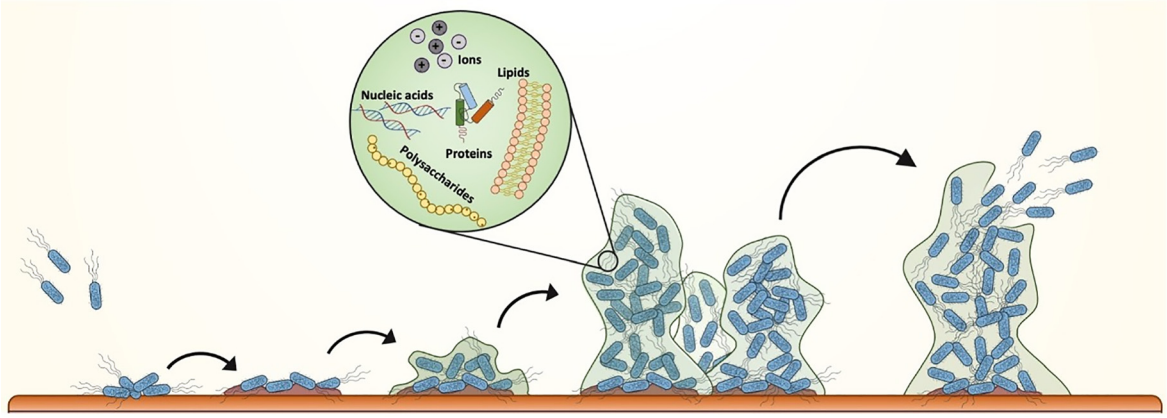


**Figure 2:** Treatment with nanoparticles has a variety of applications, including preventing, disrupting, inhibiting, or dispersing bacteria from biofilm infections.

excrete antimicrobial agents in a controlled environment to reduce the harmful effect while also increasing antimicrobial efficiency. Using NPs not only targets antibiotic delivery but also improves bioavailability [56]. Figure 2 depicts the formulation and treatment of nanoparticles for biofilm-related problems [57–68].

## 5 Factors affecting microbial toxicity

Scientists are investigating the relationship between nanoparticles and microbes in order to determine the toxic effects of nanoparticles and how they relate to physical and chemical properties. Some of the properties of nanoparticles, such as physical properties, are thought to be interrelated, while irregular changes in structure, appearance, and surface coating can alter communication with biological systems. The biological impact of nanoparticles can also be influenced by other factors such as nanoparticle synthesis, dose, additive presence and absence, material solubility, and microbe internal properties.

## 6 Parent material

The nature of the parent materials determines microbial toxicity; the relative toxicity of one material in comparison to another varies due to differences in structure, size, surface coating, and synthesis method, all of which affect toxicity. Physical properties are difficult to control, whereas changes in surface coating can be caused by different synthesis methods and accidental toxic materials. Certain materials added during the manufacturing process, such as detergents, chemicals, and additives, remain within the product and are not completely eliminated, making the product toxic. According to the literature on engineered Ag nanoparticles on bacterial efficacy with specific surface coating and size using various techniques, solvent chemical usage may lead to the formation of false toxicity. For instance, in the Ag-resistant *E. coli* was used, which led to the death of Ag-resistant bacteria due to the use of formaldehyde remnants. As a result, it has been demonstrated that the biological properties of nanoparticles are dependent on the specific parts used in formulation or the one that differs from the rest during the chemical coating process on the nanoparticle [69]. Metal ions, which are required by living organisms in small amounts, can be toxic if used in high concentrations. It has also been reported that some metals have a low solubility rate in aqueous medium, and microbes face a challenge once the metal nanoparticles convert into ions [70, 71]. The mechanism of toxicity at the molecular level differs for different ions and species, and once the nanoparticle breaks up into ions, the nanoparticle may become toxic. The toxic effects of metal ions such as copper, silver, zinc, and nickel have long been recognized as a result of microbial toxicity. Meanwhile, the nanoparticles formed as a result of these ions have become toxic. Silver is a metal ion that is commonly used as a microbial toxic material, and much research has been done on the use of silver as an antimicrobial agent, either alone or in combination [72]. Previous research has also shown that the use of silver nanoparticles during breakup can have a toxic effect. The toxicity of nanoparticles and their dissolved ions has been observed in their other materials. It is also believed that some of the nanoparticles composed of iron, gold, palladium, silver sulfide, and platinum are nontoxic in nature. Because of their low solubility in aqueous media, their metal ions are nontoxic in nature. Based on the preceding

discussion, it is concluded that the breakdown of nanoparticles into ions can result in toxicity. The generation of ROS is another reason for nanoparticle microbial toxicity. Some physical and chemically engineered nanoparticles have redox active surfaces and can react with oxygen at the molecular level to produce ROS, which are responsible for the toxic response of biological systems. Whereas metal or metal oxide nanoparticles have the ability to release ions, causing the ions to be toxic and also generating ROS, which is responsible for the toxicity of nanoparticles, the toxic effect of nanoparticles still requires further research due to unclear results [69].

## 7 Size and shape

The size and shape of nanoparticles are important factors that influence their toxicity. Size and shape are inversely related because reducing particle size increases surface area, which causes changes in the physical and chemical properties of the nanoparticle. As a result, toxic nanoparticles with smaller particle sizes form. The best examples are silver and zinc oxide nanoparticles, which are formed as particle reactivity increases. Other practical examples show that when nano-silver on nano-silica particles interacts with *E. coli*, size-dependent Ag ions are released. When the dimension of the fine particle to be used is less than 10 nm, the Ag+ is considered to be prominent. Another factor that can influence the properties of nanoparticles is shape. Particles with irregular shapes, rough and uneven surfaces exhibit approximately 10 antimicrobial activations, and it is also believed that the corners and edges (180) remain chemically and biologically active. The weak coordination of atoms at these sites has a significant impact on microorganism interactions. Different shapes of nanoparticles are engineered and commonly used by using different methods of synthesis, including rods, spheres, pentagons, triangles, squares, and hexagons. Certain literature has also supported the increased toxicity of a material due to the presence of highly reactive nanoparticles at its edges. For example, silver nanoplates in triangular shapes are thought to be more reactive against *E. coli* than particles in other shapes, such as spherical and rod-shaped [73].

## 8 Concentration

Higher concentrations of nanoparticles exhibit potent antimicrobial activity. As it performs various functions on the same microbial growth, such as mitochondrial dysfunction, increased enzyme activity (lactate dehydrogenase execration from the cell), and increased nanoparticle effect. Furthermore, a larger surface area can be covered by using a higher nanoparticle concentration, resulting in increased antimicrobial activity [74].

## 9 Roughness

Another factor that can influence the properties of nanoparticles on bacterial cells is roughness. As the roughness of a nanoparticle increases, so does its size and surface area-to-mass ratio, which increases bacterial protein adsorption and decreases bacterial adhesion [75–77].

## 10 Zeta potential

The zeta potential of nanoparticles has also been shown to improve bacterial adhesion. Nanoparticles have a positive charge, whereas bacteria's cell membranes have a negative charge, which electrostatically attracts each other. Because of these positive charges, nanoparticles are prone to being adsorbed on the bacterial cell and are closely connected [78]. In contrast to the –ive charge, neutral charge, and +ive charge, they are thought to have been used to boost ROS production. According to recent literature, nanoparticles with negative charges do not adhere to bacterial membranes because they carry the same charge. Using higher concentrations of nanoparticles with –ive charges, antibacterial activity is demonstrated due to molecular crowding, demonstrating the connection between the bacterial surface and nanoparticle [79].

## 11 Doping modification

The goal of doping medication is to avoid nanoparticle clustering and thus allow it to remain diffuse within an aqueous or other hydrophilic solvent medium. By doping, the communication between nanoparticles and bacterial cells can also be effectively controlled and regulated in a specific manner. Combinations of ZnO and Au nanoparticles form a noncomposite compound called ZnO/Au, which not only increases ROS generation but also improves photocatalytic activity. These findings improve the following factors: increased light absorption due to Au wavelength; zinc oxide width changes with band gap, increasing the reactivity of photo-induced charge carriers; and improved separation of charge carrier and electron

transport. The antibacterial activity changes as a result of doping modification. It is concluded that nanoparticles of zinc oxide doped with fluorine generate more ROS than zinc oxide nanoparticles alone, resulting in the death of bacterial cells. The O content at the surface of ZnO is thought to be the key factor in regulating antimicrobial activity against both gram-negative and gram-positive bacteria [80].

## 12 Surface coating

The nanoparticle is thought to be surrounded by a member or shell that acts as a reactive or stabilizing agent after modification. This member (surface) is regarded as a primary factor in determining a nanoparticle's environmental and biological fate because it is an important component of contact. Surface coating can affect the charge on nanoparticles, which in turn can affect the material's affinity. The surface charge of silver nanoparticles is thought to be the most important factor in their toxicity in nature. Different surface coatings were investigated, with results ranging from extremely positive to extremely negative, demonstrating that decreasing toxicity also reduced particle size [81]. Ion dissolution or release from nanoparticles is also caused by surface coating.

## 13 Particular properties of microorganisms

The effect of nanoparticles on microorganisms should be studied because they should not be similar. Several studies have been conducted to determine the response of nanoparticles to various bacterial species. It is estimated that approximately 10 antimicrobial nanoparticles activated have exerted 182 physiological characteristics on microbes that influence their growth and tolerance level to nanoparticle-induced stress. Different species of microorganisms react differently to toxic nanoparticles. Synthesized Ag nanoparticles were applied to gram-negative and gram-positive bacteria and compared, revealing that gram-negative *E. coli* and *Shewanella oneidensis* are more resistant than gram-positive *Bacillus subtilis*. Other studies on the susceptibility of gram-positive and gram-negative bacteria to nanoparticles have also been conducted [69]. According to the literature, gram-positive organisms are more sensitive to toxic nanoparticles due to their sensitive nature; this increased sensitivity is also due to differences in the cell membrane and cell wall of bacteria. Gram negative bacteria, on the other hand, are resistant to nanoparticles due to the presence of lipopolysaccharides on the bacterial cell's outer membrane [82–84]. In a study comparing the toxicity of CdTe QDs to gram-negative and gram-positive bacterial strains, the gram-positive organism was found to be more sensitive than G –ve. The release of heavy metal ions was also observed; the formation of free hydroxyl radicals is a major cause of toxicity [85]. The sensitive nature of the QDS of gram-negative bacteria over gram-positive bacteria is debatable. Certain nanoparticles have varying degrees of toxicity to different microbes. Due to the lack of a lipopolysaccharide membrane, $TiO_2$ and $Ag–TiO_2$ nanoparticles have been shown to be more toxic to *B. subtilis* than *P. putida* [71]. Bacterial tolerance to nanoparticles improves as the number of bacterial cells increases. It was also discovered that bacteria that grow faster are more susceptible to antibiotics and nanoparticles than bacteria that grow slowly and steadily [86]. The tolerance properties of slow growing bacteria can be linked to the expression of stress response genes [87]. *B. subtilis* and *P. putida* are also capable of acquiring nC60. *P. putida* has the ability to reduce unsaturated fatty acid levels while increasing cyclopropane fatty acid levels, whereas *B. subtilis* has the ability to increase transition temperature and fluidity in the presence of nC60. These properties have been shown to protect bacterial membranes from oxidative stress. *S. oneidensis* MR-1, for example, provides a practical example of how much it aids in resistance to $Cu^{2+}$ and Cu-doped $TiO_2$ nanoparticles [88], which is due to the formation of EPS under nanoparticle stress. As it mops up nanoparticles on the cell surface, this bacterium can reduce the concentration of Cu ions in the media. These bacteria are also important because they are in charge of detoxifying metal oxide nanoparticles from the environment. *Cupriavidus metallidurans* CH34 and *E. coli are* normally internalized by compounds such as $TiO_2$ and $Al_2O_3$ nanoparticles, whereas these nanoparticles are toxic only against *E. coli* [89]. The mechanism of resistance of *C. metallidurans* CH34 is still being studied. The bacterial tolerance mechanism mentioned above may be related to physical properties of their peptidoglycan layer and genes found in plasmids that have the ability to stabilize the plasma membrane of nanoparticles. Some bacteria are also capable of tolerating nitric oxide (NO) nanoparticles [73]. *E. coli*, *Pseudomonas aeruginosa coli*, and *Salmonella typhimurium* are two examples of such bacteria that are responsible for DNA repair and metal homeostasis changes in the presence of no nanoparticles. K is responsible for the production of the enzyme flavohemoglobin. Pneumonia, which counteracts nitrosative stress, furthermore, some microbes have the ability to produce biofilm, which serves

to protect them from harm. Biofilms are thought to be complex 3D microbial colonies that form by adhering to a solid surface and secreting a matrix (EPs) that serves to protect and cover the bacterial cell colony. When Ag nanoparticles are exposed to biofilm, they may inhibit the growth of new bacterial colonies and thus the development of biofilm [90].

## 14 Environmental conditions

The effect of antimicrobial activity can be altered by a variety of environmental factors. The generation of ROS is an example of how environmental temperature affects antibacterial activity. Temperature is used to restore zinc oxide (ZnO) nanoparticles and thus electrons at the targeted site. Meanwhile, ROS is produced as a result of the electron-oxygen correlation, which effectively enhances the antimicrobial effect of ZnO nanoparticles. Furthermore, other environmental factors such as pH increase the effect of *in vitro* antimicrobial activity. The level of suspension rate of ZnO nanoparticles increases as the pH decreases. Whereas the mechanism of dissipation of Ag nanoparticles has also been proposed following the interchanging of Ag+ with oxygen and protons, Variation in aquatic chemistry causes the activation of Ag nanoparticles, which release Ag ions as a result of increased antibacterial activity. According to the findings of the study, nanoparticles are more soluble in acidic water (acetic acid) than in neutral water. pH and osmotic pressure, for example, have an effect on the surface charge, clustering, and solubility of nanoparticles. Antibacterial tests on ZnO nanoparticles were performed in five different media, indicating that their activity may be due to free Zn ions and zinc complexes. It is also concluded that the medium is responsible for supplying nutrients to bacteria in order to improve their tolerance to Nps. The antibacterial effect can also be controlled by various techniques for producing ZnO nanoparticles [80].

## 15 Persister cell formation

ppGpp is associated with persistence, as there is a close agreement on alarmoneppGpp for the generation of persisters [91–94]. However, it is unknown what mechanism the ppGpp uses to form the persister cells. Understanding the mechanism that slows the metabolism rate of ppGpp will aid in understanding the relationship between ppGpp and persistence. It occurs as a result of the following factors: weather stress, cells slowing replication, transcription, and translation by synthesizing guanosine tetraphosphate and guanosine pentaphosphate [95]. ppGpp is also responsible for inhibiting DNA primase, which reduces DNA replication and transcription [95], as well as stimulating RpoS (sigmaS, the stress response sigma factor for the stationary phase) and RpoE (sigmaE, the stress response sigma factor for misfolded proteins in the periplasm) [96]. Purine nucleotide formation and purine homeostasis regulation were prevented by ppGpp through the action of the nucleosidase PpnN [97]. With a decrease in ribosome production, ppGpp translation rates also decrease [98].

ppGpp is also directly responsible for protein activity reduction, as it prevents and binds GTPases and GTPaseHflX [95]. It also binds to the protein GTPase HflX, which activates 100S ribosomes [99], and its property is to prevent inactive ribosomes from being reactivated. Furthermore, ribosomes that are linked to GTPase participate in the biogenesis of ribosome subunits, which is inhibited by ppGpp [15].

In the formation of precursor cells, ppGpp is used to inhibit the ribosomes in a variety of ways, including persuading rmf [100], which functions to encode RMF, which inactivates 70S ribosomes, (ii) inducing hpf, which encodes the hibernation promoting factor (Hpf), and (iii) inducing rai (RaiA). Other factors have also been discussed, such as how ppGpp is useful in activating the toxin system, which leads to persistence [101–103]. The use of a simpler ribosome dimerization persister (PRDP) model is being considered, which functions to generate persister cells directly via ppGpp without the use of TA systems, which convert 70S ribosomes into inactive 100S ribosomes as the ribosomes are inactivated [18, 19]. Using this model, it was determined that [19] i) ribosomes in presister cells are mostly inactive, just like 100S ribosomes. (ii) Inactivation of RMF, Hpf, and RaiA leads to the formation of persister cells, as well as an increase in single persister cell regeneration. (iii) ppGpp levels have no effect on single cell persister formation. This model has no effect on the TA system for persister cell formation because its link to persistence is unconvincing [104, 105]. Persistence occurs at lower levels of ppGpp, but as levels decrease [93], the model PRDP will behave like the cAMP function to idle Hpf and RMF, resulting in the generation of 100S and stagnant ribosomes. Famine (for example, glucose depletion) causes an increase in cAMP, prompt rmf [98], and raiA [106], while HflX is also suppressed by cAMP [107]. The cAMP model, like the ppGpp model, played a similar role in the generation of persister cells, with increased cell signal concentrations leading to inactivation of ribosomes and persistence.

## 16 Treatment for persister cells using nanoparticles

Different types of metallic NMs use different tools to destroy microbial growth and thus prevent resistance patterns. Gold (Au), silver (Ag), zinc (Zn), copper (Cu), magnesium (Mg), and titanium make up the NMs (Ti). The combined use of Bismuth NM's and X-rays is thought to be beneficial against bacteria that resist drug effects [108, 109]. It should be noted that $Al_2O_3$ NMs are thought to be free of drug resistance among metallic-NMs [110].

## 17 Application of silver nanoparticles

Ag nanoparticles perform different antimicrobial properties and are also responsible for suppressing microbial resistance in terms of growth. When silver is added to an aqueous solution, silver ions with a positive charge (Ag+) form, making it antimicrobial against a variety of bacteria [111]. The antimicrobial property of the Ag+ ion can be used in a variety of ways [112]. Initially, silver ions retaliate with groups of sulfur and phosphorous that carry proteins in the bacterial cell membrane and cell wall. Because the cell contains both negative and positive components, the silver ion attached to the negative part of the cell membrane creates a crack or hole within the membrane, allowing the cytoplasm content to escape. The cell's demise is also caused by the gradient of hydrogen ions passing through the cell membrane. If this coordination between ions and cells does not occur, the Ag+ ion will pass through the cell membrane into the cytoplasm, and the cell wall will be responsible for the stronger action of the silver ion against bacteria. Because gram-negative bacteria are more sensitive to Ag+ ions than gram-positive bacteria, Ag+ ions are more effective against them. This sensitivity of bacteria may be due to a weak cell wall, which allows Ag ions to enter the bacterial cells [90]. Furthermore, these bacteria (gram +ive) are easily harmed by these ions (Ag+ ions) because they bind to the negatively charged LPS of gram negative, whereas gram positive contains peptidoglycan, which carries a positive charge. As a result, it was concluded that Ag+ ion, when treated against gram-positive and gram-negative bacteria, was less likely to probe gram-positive cells due to LPS [113].

Silver ions are also responsible for the formation of spume within bacterial cells [115]. Other responsibilities include: 1) preventing cytochrome from being removed from electron transport in microbes. 2) Completely eradicate microbial DNA and RNA. 3) Impaired cognitive microbes' ability to copy DNA causes cell division to be delayed. 4) the presence of 30S ribosomal subunits, which suppress protein expression. 5) the formation of reactive oxygen species (ROS), which are extremely hazardous to eukaryotic cells and bacteria [116]. 6) Is also in charge of the production of gram-positive bacteria cell walls.

Ag-NMs have the ability to combat drug-resistant fungi, viruses, and bacteria, as well as some other broad-spectrum microbes. According to the literature, Ag NMs have bactericidal action against MDR bacteria such as *P. aeruginosa*, erythromycin-resistant *S. pyogenes*, and *E. coli* resistant to ampicillin [114, 117]. The effect of bactericidal drugs on various bacteria was studied, and the results show whether they are drug-resistant or drug-sensitive against bacteria. It was concluded that the fact that a protein exhibits antibiotic resistance does not change its susceptibility to Ag-NM [118]. The combination of antibacterial drugs and Ag NMs improves the antibacterial effectiveness of drugs used against *E. coli* and *S. aureus*, such as penicillin G, clindamycin, amoxicillin, vancomycin, and especially erythromycin. Wang et al. investigated the effect of Ag-NMs in conjunction with antibiotics such as levofloxacin, revealing a synergistic action with a favorable safety profile while studying animals [119]. This study also discovered that NMs of silver carbene complex wrappers were toxic to MDR bacteria [104]. According to the study, Ag-NM also has antiviral properties against HIV and HBV [120].

## 18 Zinc oxide nanoparticles

Zinc nanoparticles are used in a variety of applications to make microbes more resistant [121]. The medium in question is 1) widely used by others. Bacterial membranes are destroyed when they are strongly connected with zinc nanoparticles. As a result of the membranes being punctured, the cytoplasmic content is released from the cell, causing the cell to die. 2) When nanoparticles puncture bacteria cells, they produce zinc ions and reactive oxygen species (ROS) along with hydrogen peroxide ($H_2O_2$). When used in high concentrations, zinc nanoparticles have been shown to be toxic. It has antibacterial properties against MDR bacteria such as MRSA and methicillin-resistant bacteria [122, 123]. Pati et al. detected the existence of *S. aureus* in mice treated with ZnO NMs. Infection was caused in mice via the interadermal (ID) route, and zinc nanoparticles were used to treat the infection on the same day (*S. aureus* + zinc nanoparticles) or one day later.

## 19 Copper oxide nanoparticles

CuO nanoparticles have been used in two ways to combat microbial resistance [124]. 1) Within the microbial cell, Cu reacts with amine and carboxyl groups. CuO nanoparticles are effective against microbes with a high density and those with a group on the cell surface, such as *B. subtilis*. 2) Because of the increased concentration of copper ions, ROS are produced, which impedes amino acid synthesis and DNA replication within microbial cells [125, 126]. CuO-NPs have a weaker antibacterial effect but a stronger microbicide action against fungi. *S. cerevisiae* and other microbes such as *Listeria monocytogenes*, *E. coli*, and *S. aureus* are examples. This microbicide action is dependent on the shape and dose, i.e., the effect improves as the dose of CuO-NPs increases [127, 128]. The figure below depicts the destruction of copper nanoparticles (Figure 3).

## 20 Titanium dioxide nanoparticles

Titanium dioxide nanoparticles use two methods against microbes, and the results show that $TiO_2$-NPs have less resistance. The following are included in the systematic tool: When exposed to UV radiation, $TiO_2$, which contains OH and $H_2O_2$ free radicals, generates ROS in a photocatalytic process. When $TiO_2$ approaches microbes, the bacterial cell becomes punctured due to ROS, understanding membrane acceptability, encroaching oxidative-phosphorylation, and producing cell disfigurement [130, 131]. 2) Even if the cells have not been treated against irradiation, $TiO_2$-NPs still exhibit bactericidal activity and other antimicrobial activity. Liu et al. noted that $TiO_2$ 101 and 001 contain valence bands and stunned conduction. The formation of a 101–001 surface heterotransition can produce quick photo electrons capable of transferring from (001) to (101), whereas the mess proceeds in another direction, resulting in a 3D separation of the electron-dump. In comparison to $TiO_2$-NPs, they generate a lot of ROS and have a lot of antimicrobial action. In the test against *E. coli* and *S. aureus*, $TiO_2$ NPs were used in the test, and their responses were recorded in the presence of sunlight and compared to controls [132].

## 21 Magnesium nanoparticles

Mg-NMs are made up of Mg halogen NPs (MgX2-NPs) and magnesium oxide NPs (MgO-NPs). These NPs are used in a variety of antimicrobial applications; their resistance is suspected in the following ways: 1) When metal halides are used, the enzymes in the bacterial cell are squeezed. 2) MgX2 is responsible for the generation of ROS, which results in the peroxidation of lipids in microbial cell

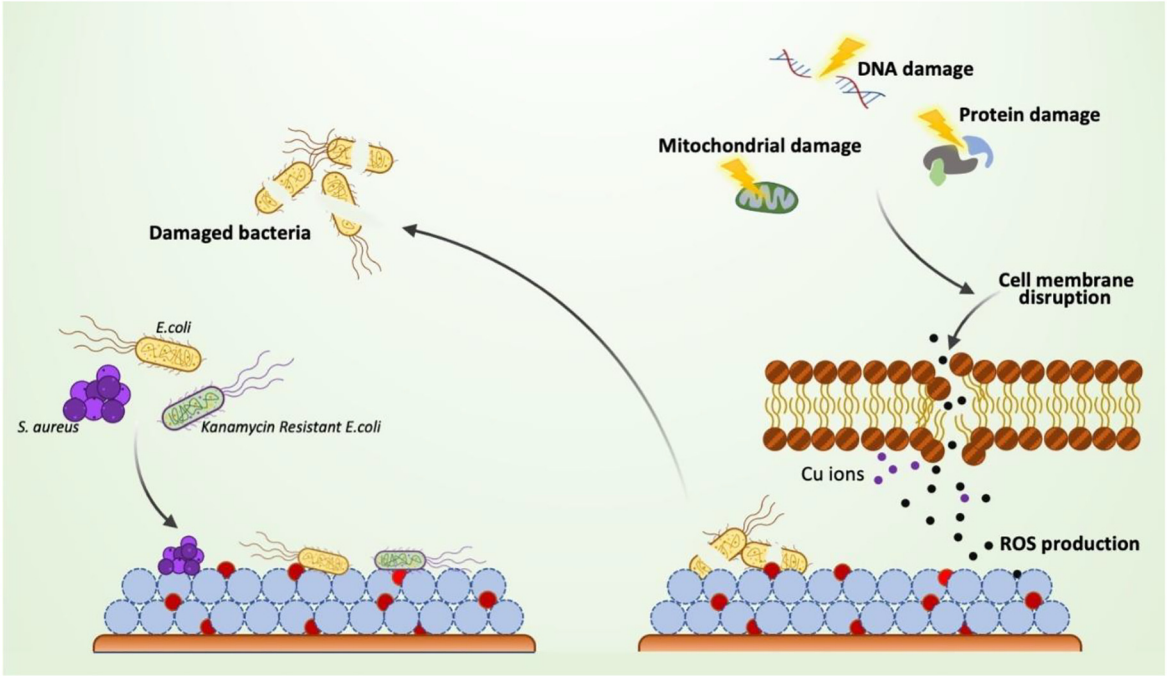


**Figure 3:** The destruction of bacteria by application of Cu-NP incorporated MI-dPG surface coating via the "attract-kill-release" route. Copyright (2017) American Chemical Society [129].

membranes, and thus targets the cell's cytoplasmic content [133]. 3) MgF2-NPs improve the lipid peroxidation process that occurs across the membrane of microbes (cells), resulting in a decrease in the pH of the cytoplasmic content and thus improving membrane capability. MgF2-NPs inhibit the production of biofilm by *E. coli* and *S. aureus* [134]. 4) Mg oxide inhibits microbes by attracting halogen molecules to the MgO surface. Because of its adsorption in MgO, wrapping Mg oxide in MgO-NPs increases the number of halogen molecules by up to five times, resulting in increased halogen action [135]. The activity of MgO NMs against *Bacillus* and *E. coli* increases due to $Cl_2$ and $Br_2$ communication with MgO-NMs, whereas this activity is lower for *B. subtilis* spores [136].

## 22 Gold nanoparticles

They are created through the use of various techniques, as previously demonstrated by research [137, 138]. Au-NPs do not have antibacterial activity on their own, but when combined with other antibiotics, they enhance their antibacterial properties [135, 136]. Brown et al. treated ampicillin binds to the surface of gold-NPs (Au-NPs-AMP) and terminates drug resistance in bacteria such as *MRSA*, *E. coli*, *Enterobacter aerogenes*, and *P. aeruginosa* [93]. When combined with antimicrobial substances [139], Au-NMs have a variety of properties that help to activate and increase antimicrobial activity when combined with them [140, 141]. Antibiotics (kanamycin and levofloxacin) in combination with Au-NPs upturn the activity of anti-bacterial [142, 143]. According to the literature, bacteria that lack the ability to endocytosis do not take AuNPs. Antibiotics (ampicillin) that inhibit cell wall function pass through the walls of G +ve and G –ve cells to provide antibacterial activity. The presence of ampicillin on Au-NMs allows NPs to enter bacterial cells. Scientists are still looking into two methods that worked together to kill bacteria. The presence of ampicillin molecules on the outer portion of Au-NM allowed Au-NM-AMP to inhibit lactamases. Furthermore, AuNPs, which extrude drug molecules from bacterial cells, obstruct transmembrane pumps [144].

## 23 Aluminum oxide nanoparticles

$Al_2O_3$-NPs are a type of metallic NM that has the ability to reverse drug resistance. According to the study [145], the $Al_2O_3$-Nps can also invade the cytoplasm of *E. coli* to cause a toxic effect. The higher the dose of $Al_2O_3$-Nps, the more it splits the cell walls of bacteria, but it also has a negligible effect on bacterial growth [146, 147]. This study also found that conjugating $Al_2O_3$-Nps with *E. coli* to *salmonella* increases the risk of antibiotic gene transmission by up to 200 times. It was determined that the bacterial gene is more resistant to one or more drugs. Qiu et al. reported that $Al_2O_3$-Nps causes the oxidative breakdown of bacteria's cell membrane, which causes an increase in gene expression countersign communication and a decrease in gene inhibition coexistence.

## 24 Future prospective

The preceding studies demonstrate that nanoparticles can prevent and control biofilm formation. Some of the research looks into their mechanisms *in vivo* and *in vitro*. However, there are some challenges, such as biosafety, biocompatibility, adverse effects of systemic toxicity, target area, and nanoparticle concentration, which may be harmful to humans, plants, and animals. More research is needed to determine the toxicity of these nanoparticles in both terrestrial and aquatic environments.

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