International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Potential Applications of Nanoparticles for Combatting Antibiotic-Resistant Oral Bacteria

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Abstract**—** *The oral microbiome, like gut microbiota, have strong resistance and are the cause of periodontal disease. Biofilm is a means of resistance that is drawing attention most recently. Existing super-strong antibiotics alone cannot destroy biofilm. Nanoparticles are emerging as a new alternative. In particular, metal nanoparticles can directly attack the biofilm components and destroy planktonic bacteria's cell walls.*

Keywords**—** *Oral Microbiome, Oral Cavity, Biofilm, Nanoparticles, Extracellular Polymeric Substances, Horizontal Gene Transfer, Gram-Positive Bacteria, Gram-Negative Bacteria, Peptidoglycan, Polysaccharides.*

I. Introduction

Nanoparticles are attracting attention as a new alternative that can eliminate resistant bacteria. There are about 700 kinds of bacteria in the oral cavity[1]. The oral microbiome refers to the microorganisms found in the human oral cavity[1]. Oral microbes cause *Periodontitis* and other dental diseases[2]**.** Many research papers are analyzing how nanoparticles can kill oral bacteria by overcoming antibiotic resistance. There are two main ways in which nanoparticles kill bacteria. One is to destroy biofilm, and the other is to penetrate the cell wall, preventing replication and transcription of bacteria[2]. Research on how to destroy cell walls have two pathway. One is to destroy the cell wall by inducing chemical interaction in the cell wall[3]. The other is to cause physical destruction of the cell wall by allowing nanoparticles to accumulate on the cell wall[3]. As described above, concerning the method of killing bacteria, a follow-up study was also conducted on which material nanoparticles produce optimal efficiency. For example, we also reviewed the optimal conditions for metallic nanoparticles according to their size and shape. This study aims to find a conventional treatment using nanoparticles by analyzing many research papers on removing oral bacteria by nanoparticles.

II. Survival Strategy of Oral Microbiome

Oral bacteria have similar survival strategies to the gut microbiome. Resistance and proliferative capacity are the two central axes for survival. Bacteria have various mechanisms for resistance to antibiotics. The most fundamental resistance is the breakdown of drugs in the cytoplasm or release of drugs into the extracellular matrix[4]. Bacteria usually exist in the form of individual cells. However, all bacteria live in a collective state. Bacteria exist as Planktonic cells theoretically but exist in the formation of biofilms[4]. All bacteria form a biofilm and are resistant to antibiotics[5]. However, the shape of the biofilm is different for each type of bacteria. In particular, biofilms between gram-positive bacteria and biofilms between gram-negative bacteria are other[6]. When many bacteria gather at a point in the oral cavity, they release extracellular polymeric substances (EPS) to create a biofilm quickly[6]. Biofilm is the most robust means of resistance to antibiotics. Biofilms represent a protected mode of microbial growth and confer significant survival advantages in hostile environments[6,7]. Thus, biofilm-forming organisms show increased resistance to antibiotics, either due to decreased penetration of the antibiotic through the biofilm matrix or the expression of more complex biofilm-specific resistance mechanisms[7]. Then, the growth and proliferation of bacteria and extracellular polymeric substance (EPS) sets in[8]. A few hours later, the biofilm development may be complete already, providing bacteria perfect protection to proliferate[8]. After the complete formation of a biofilm layer, individual biofilm fragments release, and the microorganisms in the protective matrix contaminate the disinfection solution[8]. Depending on the bacterial strain, the physicochemical properties of the biofilm are different[9]. The biofilm formed by bacteria interacts dynamically and builds a stronger position[9]. The biofilm helps the bacteria to adhere firmly to each other[9]. Simultaneously, the biofilm provides a physical space to maintain close distances from each other by limiting newly grown bacteria's movement[9]. Biofilm is a means of protecting bacteria against antibiotics, and it also functions as a platform for Horizontal gene transfer(HGT)[10].

III. The Gram Strain Protocols

In bacteria, the Gram stain provides a vital classification system, as several cell properties correlate with the cell envelope[11]. Gram-positive bacteria possess a thick (20–80 nm) cell wall as the cell's outer shell[11]. In contrast, Gram-negative bacteria have a relatively thin (<10 nm) layer of cell wall but harbor an additional outer membrane with several pores and appendices[11]. These cell envelope differences confer different properties to the cell, particularly responses to external stresses, including heat, UV radiation, and antibiotics[11].

The cell wall components of gram-positive and gram-negative bacteria are different[12]. Gram-positive bacteria have cell walls that contain thick layers of Peptidoglycan (90% of the cell wall)[12]. Gram-negative bacteria have walls with thin layers of Peptidoglycan (10% of the cell wall) and high lipid content[12]. According to the cell wall component, we are to devise the antibiotic strategy by nanoparticles differently.

Dongju Lee, "Potential Applications of Nanoparticles for Combatting Antibiotic-Resistant Oral Bacteria," *International Research Journal of Advanced Engineering and Science*, Volume 6, Issue 1, pp. 145-150, 2021.

International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Peptidoglycan is an essential component of the cell wall in Gram-positive bacteria[12]. Gram-positive bacterial cell wall-associated polysaccharides are often negatively charged (teichoic and teichuronic acids)[13].

TABLE 1. The Gram Strains in the Oral Cavity[1,3,4,7].

Oral Cavity	Gram-negative bacteria	Gram-positive bacteria
Strains	*Moraxella, Neisseria, Veillonella Campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella, Fusobacterium, Hemophilus, Leptotrichia, Prevotella, Selemonas, Simonsiella, Treponema, Wolinella.*	*Abiotrophia, Peptostreptococcus, Streptococcus, Stomatococcus Actinomyces, Bifidobacterium, Corynebacterium, Eubacterium, Lactobacillus, Propionibacterium, Pseudoramibacter, Rothia.*
Cell Wall	Lipopolysaccharides(90%) Peptidoglycan(10%) (thin)	Peptidoglycan(90%) Polysaccharides(10%) (thick)

Teichoic acids are cell wall-associated macromolecules, such as polyols or carbohydrates[13]. Usually, such basic structures are further substituted by various sugars and amino acids[13]. In general, teichoic acids are connected to muramic acid of Peptidoglycan via a phosphodiester bridge[13]. In Gram-positive bacteria, the cell wall thickness varies from 20 to 40 nm[14]. It functions as a protective barrier against the external environment[14]. The cell wall's principal component is Peptidoglycan, which also serves as a scaffold for attaching proteins and polysaccharides[14]. The Gram-negative cell wall is composed of an outer membrane, a *peptidoglycan* layer, and a *periplasm.* In the Gram-negative Bacteria, the cell wall comprises a single layer of Peptidoglycan surrounded by a membranous structure called the outer membrane[14]. The Gram-negative cell wall is thinner (10 nanometers thick) and less compact than Gram-positive bacteria[15]. Still, it remains strong, challenging, and elastic to give them shape and protect them against extreme environmental conditions[15]. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS)[15].

IV. Nanoparticles, Novel Antibacterial Agents

Nanoparticles are primarily classified into metals and nonmetals. Gold and silver are typical metallic nanoparticles. Gold and silver nanoparticles have several advantages: a high surface area to volume ratio, amenability to surface modification, small size (less than 10 nm), and static nature[16]. These advantages make metallic nanoparticles the most suitable choice for drug delivery and antibiotic therapy[16]. The antibiotic function of the nanoparticles, as expected, is their attack power against the biofilm. Metal nanoparticles are attracting the most attention recently because of their potential for biofilms. Nanoparticles must cross the biofilm wall before accessing bacterial cells[17]. Nanoparticles interact with bacterial cell membrane components depending on their surface chemistry, charge, and hydrophobicity[17]. The composition of the biofilm is different depending on the type of bacteria. The nanoparticles' penetration power depends on the biofilm's maturity, constituent materials, surface tension, size, concentration, and shape[18]. Nanoparticles go through three steps: approaching, penetration, and moving inside the biofilm[18]. In this process, electrostatic, hydrophobic, hydrogen-bonding, and Van der Waals forces work[18].

Nanoparticles that enter the biofilm space must re-enter the bacterial cells. Bacteria have a rigid cell wall. Nanoparticles interact with the lipid bilayer and LPS[19]. As a result, it induces the bacterial cell membrane's fluidization and destroys the bacterial cell membrane[19]. Nanoparticles on the bacterial cell membrane release ions. The released ions enter the cytoplasm through pores in the cell membrane[20]. Nanoparticles change the structure of cell membrane proteins. Ions attack efflux pumps proteins, one of the means of antibiotic resistance, and neutralizes resistance[20]. The attacks on cytoplasmic structural proteins continue[20]. When these phenomena accumulate, the bacteria eventually interfere with their metabolism, leading to death[20]. Nanoparticles are most effective when attacking the biofilm first and then bacteria. When the signal exchange between bacteria is blocked, the bacteria in the group form become individual units of Planktonic cells[21]. Ions released from metal nanoparticles produce ROS in bacterial cell membranes[21]. Superoxide Radicals, Hydroxyl Radical, and Hydrogen peroxide are typical ROS[21]. These radicals increase the permeability of bacterial cell membranes[21]. Most notably, ions from nanoparticles interfere with the electron transport system of bacterial cells[22]. As a result, bacteria cannot metabolize cell membranes normally, resulting in increased permeability, resulting in unstable cell membranes[22]. Eventually, metabolic disorders occur in the cytoplasm, leading to death[22].

V. The Nanoparticles, Anti-Biofilm Activities

Biofilms are made of a variety of materials. It isn't easy to obtain the desired effect because the nanoparticles non-specifically act on the biofilm[23]. By analyzing the materials that make up the biofilm, it is necessary to attack the molecules that play the most critical role in the biofilm[23]. eDNA occupies the largest proportion in biofilm[24,25]. Unlike eukaryotic cells, bacteria do not have DNA inside the nuclear membrane. In principle, eDNA exists in the cytoplasm, and it also exists in the extracellular matrix[24,25]. DNA present in the extracellular matrix is called eDNA[24]. DNA molecules are not found exclusively within cells but are an essential component of the extracellular medium. Extracellular DNA (eDNA) has long been known as one of the most abundant molecules in slimy biological matrices[26]. Moreover, eDNA has been revealed as a critical component of the extracellular matrix of multicellular communities[26]. Most known eDNA release mechanisms are regulated by quorum sensing (QS): a cell density-dependent communication system that governs cooperative behaviors[26,27]. Therefore, eDNA is usually produced in response to an increase in the cell density of the population[24]. Besides, it is noteworthy that in several bacteria, the eDNA release pathways are related to natural competence development, enabling the cells to be transformed by DNA eDNA is directly involved in biological roles, biofilm formation, structure, and integrity[26,27,28].

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eDNA is the result of bacterial killed following the use of antibiotics[26]. Even if the bacteria die, their DNA is not destroyed, but moves to the extracellular matrix, eventually leading to eDNA[26,27]. eDNA has no mechanism to produce protein[24]. However, eDNA combines with lipids and proteins in the biofilm to form a single solid mass[25]. Metal nanoparticles can effectively bind to eDNA, weakening the structure of the biofilm[28,29]. Of course, here, the Van der Waals force, hydrophobicity comes into play[29,30]. When the metal nanoparticles remain tightly bound to the eDNA, the robustness of the biofilm is destroyed[28,29]. Ions released from metal nanoparticles bind to proteins in the biofilm[30]. It is called molecular docking[30]. Among the amino acids that are constituents of proteins, aspartate binds to each other by static electricity and tyrosine by hydrophobic action[31]. Many proteins present in the biofilm play a key role in Quorum Sensing (QS), which keeps the number of bacterial populations constant[31,32]. Proteins cannot maintain their three-dimensional structure by hydrogen bonding, hydrophobic bonding, and electrostatic bonding between amino acids, components of specific proteins, and nanoparticles[33].

Proteins with modified structure cannot function as a ligand for QR[33,34]. It is difficult to maintain the number of bacteria, and the biofilm is not robust[34]. It is the result of the binding of metal nanoparticles to proteins in the biofilm[34]. The ions from the metal nanoparticles bind to proteins that make up the cell membrane of bacteria[35]. Proteins constituting cell membrane components are essential for bacterial metabolism through the electron transport system[35]. Metal ions immediately bind to proteins that make up the cell membrane and interfere with electron transfer, resulting in a weakening of the bacterial toxicity[35]. The attack of metal ions against HSP-18, which repairs the modified protein, hinders bacteria's biofilm formation[36]. In particular, positively charged metal ions and negatively charged cell membrane proteins are strongly bonded by electrostatic forces[37]. Due to this binding, the electron transport system of the cell membrane protein is broken[35,37]. There are two types of polysaccharides in biofilm. One exists in the cell wall of bacteria, and the other participates in the biofilm structure[39]. Specifically, polysaccharides are the building blocks of bacteria and substances that are secreted by bacteria[40]. Polysaccharides support bacteria with mechanical strength, structural stability, and robust defects between bacteria[40]. To attack the biofilm, we must overcome the barrier of polysaccharides. Polysaccharides serve as the best targets for biofilm inhibition strategies[41].

Metal nanoparticles have the potential to inhibit their function through interaction with polysaccharides[41,42]. In particular, they are called Lipopolysaccharides (LPS) that make up gram-negative bacteria's cell walls[43]. The bacterial cell wall has a negative charge[41]. In Gram-positive bacteria, this negative charge is the presence of teichoic acids linked to the Peptidoglycan[13,42]. These teichoic acids are negatively charged because of the presence of phosphate in their structure[43]. The Gram-negative bacteria have an outer covering of phospholipids and Lipopolysaccharides[43]. The lipopolysaccharides impart a strong negative charge to the surface of Gram-negative bacteria[42]. Metal nanoparticles on the cell wall of gram-negative bacteria create more vital electrostatic interaction[43]. The cell wall of gram-positive bacteria is composed of teichoic acid[13,44]. The positive charge of the metal nanoparticles and the negative charge of the gram-positive bacteria's teichoic acid interact to generate a weak electrostatic force[13,44,45]. The binding force between the metal nanoparticles and the Gram-positive bacteria is more vulnerable than the Gram-negative Bacteria [45]. Lipopolysaccharides (LPS) 's strong negative charge allowed the metal nanoparticles to induce a robust electrostatic force[46]. The hydrophobic properties of biofilm come from lipid, LPS, surfactants, etc.; the hydrophobicity of biofilms is mostly derived from lipids[47]. The lipid component of biofilms plays a crucial role in supporting the binding between bacteria[47]. When metal nanoparticles bind to lipids and interfere with lipids' function, they can effectively attack the biofilm[48]. Some studies have shown that a more vital hydrophobic force between the Cholesterol PEG-coated metal nanoparticles and bacteria acts to destroy the biofilm more effectively[49,50]. The negatively charged bacterial cell membrane increases the electrostatic force's bonding force with the positively charged metal nanoparticles[51]. Specifically, the hydrophobic force acts on the nanoparticle's lipid component and the biofilm, and the electrostatic force acts on the bacterial cell membrane and the nanoparticle[51]. The size and shape of the nanoparticles also have different responsiveness to the biofilm[52]. The average length of the nanoparticles is 13nm-90nm[52]. The smaller the nanoparticles, the easier it is to penetrate the bacterial cell membrane[53]. At the same time, it is of great help in interfering with bacterial resistance[53].

Bacteria maintain resistance by operating an efflux pump that releases antibiotics from the cell membrane when they enter the cytoplasm[23,30]. However, if the nanoparticles are small, it isn't easy to dismiss them by an efflux pump[23,53]. If the nanoparticles are less than 20 nm, they can quickly enter the biofilm's pores, showing practical antibiotic ability[53,54]. The shape of the nanoparticles also makes a difference in antibiotic ability[54]. Among the sphere nanoparticles, star-shaped nanoparticles, and flower-shaped nanoparticles, studies have shown that star and flower-shaped nanoparticles have better antibiotic capabilities[54,55]. The star and flower-shaped nanoparticles have a larger surface area than sphere nanoparticles and are easier to combine with the biofilm's constituent materials[54]. There are also experimental results for square and triangular nanoparticles, but round nanoparticles' antibiotic ability is the best[56]. Compared to nanoparticles emitting negative ions, the nanoparticles emitting positive ions showed the best antibacterial activity[57]. The pH concentration inside the biofilm also acts as a substantial variable in nanoparticles' antibacterial capacity[58]. At a neutral pH concentration, the nanoparticles are stable and uniformly diffuse into the biofilm[58]. However, in a highly acidic biofilm, charge inversion occurs in nanoparticles[58]. The increased electrostatic repulsion inhibits the diffusion of nanoparticles, resulting in gathering in one place[58]. Biofilm has many holes. Nanoparticles can enter the biofilm through this hole. By the

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way, the biofilm has tiny pores (10nm-20nm), which inhibits the passage of larger nanoparticles[59].

VI. Conclusion

Oral bacteria, either gram-positive or gram-negative, form biofilms. The nanoparticle plays a crucial role in destroying the biofilm. It is necessary to create a strategy to attack the biofilm by separating metal nanoparticles from nonmetallic nanoparticles[60]. After dismantling the biofilm, it is most useful to kill individual bacteria. The materials that make up the biofilm, either gram-positive or gram-negative, are almost the same. Biofilm contains polysaccharides, eDNA, protein, lipid, LPS (Lipo-polysaccharides), surfactants, etc[2,20,34]. It is an unrealistic strategy to attack biofilms by various types of nanoparticles simultaneously. It requires choice and concentration. The primary goal of nanoparticles is designed to destroy the binding force between bacteria in the biofilm[61]. It is necessary to attack the material that most strongly supports the biofilm structure. Polysaccharides help the robustness of the biofilm structure, and eDNA contributes to the maintenance of the biofilm[3,11,19]. It is most efficient to establish an attack strategy for these two materials through nanoparticles. Polysaccharides have hydrophilic properties, so they have a strong bond with water[62]. Assuming that the biofilm in contact with the nanoparticles is in the mouth, water is mixed[62]. A negatively charged oxygen atom in water molecules and a positively charged hydrogen atom have electrostatic force based on hydrogen bonds[62]. Because Van der Waals forces act between water molecules, it is difficult for nanoparticles of 50 nm or more to access the polysaccharides in the biofilm[63,64]. Nanoparticles should be designed in 20 nm or less for obtaining a powerful Van der Waals force to overcome water tension and bind to the polysaccharide[62,63,64]. Meanwhile, eDNA is also a hydrophilic polymer material. Therefore, metal nanoparticles of 20 nm or less must be inserted to overcome the hydrophilicity of eDNA[63]. Nanoparticles bound to eDNA can inhibit the production of proteins involved in biofilm formation through the HGT of eDNA[62,63].

A second strategy is needed to attack individual bacteria again while the biofilm is disassembled. A typical oral disease is a periodontitis. It is mainly gram-negative strains that cause Periodontitis[24]. Compared to gram-positive bacteria, gram-negative bacteria have a thinner cell wall. They are LPS components, so gold or silver nanoparticles of 20 nm or less can sufficiently pass through the cell wall. In other words, small-sized nanoparticles can infiltrate the cytoplasm of gram-negative bacteria and bind to proteins or DNA, interfering with metabolism.

On the other hand, gram-positive bacteria have a thick cell wall, making it difficult to pass through the cell wall even if they are small nanoparticles of 20 nm or less. The cell wall of gram-positive bacteria comprises Peptidoglycan; most of the Peptidoglycan components are occupied by negatively charged teichoic acid[42,44]. An electrostatic force acts between gram-positive bacteria with negatively charged cell walls using positively charged metal nanoparticles. Based on these interactions, it is best to attack the cell walls of gram-positive bacteria physically. Metal nanoparticles accumulate on the cell wall. The electron transport system, which was in charge of the protein inside the cell wall, is disturbed, resulting in gram-positive bacteria's death.

TABLE 2. Antibiotic strategy in the oral cavity using metal nanoparticles[64,65,66,67,68,69,70]

Oral Cavity	Biofilm	Cell Wall
Gram-negative bacteria	20nm less than	20nm less than
Gram-positive bacteria	20nm less than	50nm less than

The biofilm formed by oral bacteria is covered with water. Nanoparticles must first overcome the tension of the water for attacking the biofilm. Even if the nanoparticles with' 50 nm or more are inserted into an oral cavity, bacteria continue to grow thanks to the water molecules surrounding the biofilm. The tension of water hindered the cohesive force of the 50 nm or more size of the nanoparticles. When the nanoparticles were smaller than 20 nm, the power of pulling each other between the nanoparticles was strong under the action of the Van der Waals force. The tension (pull force) between the nanoparticles with small and circular is more robust and tends to become a single lump[67]. Therefore, nanoparticles with a circular shape of 20 nm or less are optimal when making a liquid powder type antimicrobial agent[67]. Individually attacking planktonic bacteria by nanoparticles requires a different approach between Gram-positive and Gram-negative bacteria. Gram-positive bacteria with relatively thick cell walls are suitable to induce the cell wall's chemical destruction by free radicals. Gram-negative bacteria with relatively thin cell walls are best to directly attack cytoplasmic metabolites rather than the cell wall's collapse[68]. For example, + Charged silver nanoparticles quickly contact the cell membrane of - charged bacteria. At this time, the silver nanoparticles act as a catalyst by releasing silver cationic ions. Accordingly, oxygen present in the cell membrane turns into active oxygen, and free radicals attack the cell membrane and destroy the cell membrane. For Gram-negative bacteria with thin cell walls, small-sized metal nanoparticles can penetrate the cytoplasm through pores in the cell wall. When nanoparticles bind to proteins, lipids, and DNA inside the cytoplasm, bacteria's metabolism is disturbed, leading to death[69,70].

Acknowledgments

I would like to think of all the authors mentioned in the reference.

Conflicts of Interest: The author declares no conflict of interest.

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