Disrupting Bacterial Biofilms to Combat Dental Diseases Using Nanotechnology in Oral Healthcare

Hunny Dabas[1], Faraat Ali[2], Fairy[3], Khushi Quadri[1], Manshi Dangi[1], Parween Jamil[4], Mohammed Aslam[5], Manvi Singh[1*]

Abstract

Oral biofilms, predominantly bacterial, are key contributors to dental caries and periodontal diseases, posing significant challenges to oral health management. Conventional methods, including mechanical removal and antimicrobial agents, often prove inadequate due to the inherent resilience and complexity of biofilm structures. Recent advancements in nanotechnology offer a transformative approach to biofilm disruption, presenting unique opportunities for oral healthcare innovation. Nanoparticles, with their distinct physicochemical properties, enable precise interactions with bacterial cells and effective penetration into biofilms, targeting bacteria in otherwise inaccessible areas. Their multifunctional nature facilitates the delivery of combined therapeutic agents, enhancing antimicrobial efficacy and addressing diverse bacterial strains. These properties underscore the potential of nanoparticles to overcome the limitations of traditional treatments, paving the way for targeted, efficient, and minimally invasive strategies. As research evolves, the integration of nanoparticles into oral healthcare practices promises to revolutionize the management of bacterial biofilms, offering personalized and groundbreaking solutions to improve oral health outcomes.

Significance | This review discusses the use of nanoparticles as innovative solutions for disrupting bacterial oral biofilms, enhancing antimicrobial efficacy, and revolutionizing oral healthcare with minimal resistance.

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Introduction

Oral biofilms, intricate microbial communities predominantly composed of bacteria, form on various surfaces within the oral cavity, including teeth, gums, tongue, and cheeks (Ahn et al., 2009). These biofilms confer substantial resistance to external chemicals, presenting a formidable challenge to oral health management. Chronic dental infections, such as caries, gingivitis, and periodontitis, are strongly associated with oral biofilms and can lead to systemic complications affecting the cardiovascular and digestive systems (Allaker & Douglas, 2009). Opportunistic bacteria within these biofilms exacerbate the persistence of recurring infections, underscoring the critical need for effective therapeutic strategies to mitigate oral pathogenic biofilms (Baehni & Takeuchi, 2003).

The overuse and misuse of antibiotics have accelerated the emergence of multidrug-resistant bacterial strains, posing a significant global healthcare threat (Namiki et al., 2011). The structural resilience of oral biofilms contributes to the diminished efficacy of antibiotics, creating an urgent demand for novel approaches to oral biofilm management. In recent years, nanotechnology has emerged as a transformative tool, providing innovative solutions to combat oral biofilms (Pablico et al., 2013).

Nanoparticles, defined as particles less than 100 nm in size (Aslam et al., 2022), possess unique physicochemical properties, including biocidal, anti-adhesive, and transport capabilities, making them

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promising candidates for biofilm disruption. Their applications extend across medical domains, from antimicrobial coatings for medical devices to drug delivery systems (Allaker, 2010; Lu et al., 2018). Emerging research highlights their potential in controlling biofilms, with studies demonstrating their ability to penetrate biofilm matrices and target resident bacteria effectively (Ferrer & Mira, 2016). In dentistry, nanoparticles are being explored as components in dental materials, prosthetic coatings, and topically applied agents (Wei et al., 2019).

Despite advances in understanding biofilm formation and persistence, effective treatment options remain limited (Zhang et al., 2018). Nanoparticle-based strategies offer a promising alternative by directly targeting biofilm structures and microbial components with minimal toxicity, positioning them as suitable candidates for clinical applications (Bowen et al., 2018).

This review provides a comprehensive examination of oral biofilm formation, its mechanisms, and the challenges it poses to oral health. It highlights the potential of nanoparticles as disruptors of oral bacterial biofilms, discussing their applications, mechanisms of action, and implications for future oral healthcare. By integrating insights into the dynamics of biofilms and the innovative use of nanotechnology, this article aims to advance understanding and foster the development of targeted, efficient, and minimally invasive strategies for managing oral biofilms.

2. Mechanism of bacterial biofilm formation/ life cycle:

The process of biofilm development in the oral cavity is not static; it evolves through autogenic succession. The establishment of specific bacterial species paves the way for the arrival of others, leading to a complex and dynamic microbial community. Understanding these processes is vital for comprehending the dynamics of oral health and disease, as disruptions in the balance of oral biofilms can contribute to conditions such as dental caries, gingivitis, and periodontitis (Hall and Stoodley, 2002).

The initiation of oral biofilm formation involves primary colonizers, such as Streptococcus and Actinomyces species. These bacteria are termed primary because they are among the first to attach to the salivary film covering the teeth. This initial attachment is facilitated by the acquired enamel pellicle, a thin layer of salivary proteins that forms on the tooth surface. Streptococcus and Actinomyces play a crucial role in creating a foundation for the subsequent stages of biofilm development (Rath, *et al.* 2021).

Ongoing research in this field seeks to uncover innovative strategies to manage and maintain oral health by targeting the intricate mechanisms of oral biofilm formation.

The self-generated extracellular polymeric matrix (EPS) serves as a protective covering for the microscopic organisms that make up the complex biofilm. Along with EPS, other components include flagella, sticky fibers, and even deoxyribonucleic acids, which bind carbohydrates (Di Martino, 2018). Changes in bacteria can have an impact on how biofilms grow, and several steps must be taken for the formation of biofilms.

***2.1 Initial or reversible attachment*:** The initial or reversible attachment is the first crucial step in the mechanism of bacterial biofilm formation. In this phase, bacteria in a fluid environment make initial contact with a surface. The process begins with the transport and approach of planktonic bacteria towards a suitable substrate. Physicochemical interactions, including van der Waals forces and electrostatic attractions, facilitate the reversible adhesion of bacteria to the surface. During this attachment, bacterial cells experience weak and transient interactions with the substrate, allowing them to explore and sense the environmental cues of the surface. The acquired enamel pellicle, a thin layer of salivary proteins, may precede this step, providing a conditioning film that promotes bacterial adherence. The success of the initial attachment is influenced by various factors, including the physicochemical properties of the substrate and the bacterial cell surface. The bacteria may express adhesins or surface appendages that enhance their ability to adhere to specific surfaces. Additionally, environmental conditions such as pH, temperature, and nutrient availability play a role in modulating the initial attachment. This reversible attachment sets the stage for subsequent stages of biofilm formation, providing a foundation for the irreversible attachment and further development of the biofilm matrix. Understanding this initial step is pivotal in designing strategies to intervene in biofilm formation and prevent the establishment of persistent microbial communities on surfaces in diverse contexts, including oral health and medical implants (Messi, 2013).

2.2. Irreversible attachment: The second step in the mechanism of bacterial biofilm formation is irreversible attachment, following the initial reversible attachment. During this stage, bacteria firmly adhere to the substrate, transitioning from weak and transient interactions to more stable and permanent binding. Irreversible attachment is characterized by the development of stronger bonds between bacterial surface structures and the substrate, cementing the bacteria in place. Bacterial adhesions, which are specialized molecules on the microbial cell surface, play a crucial role in this process by binding tightly to specific receptors on the substrate. These interactions may involve biochemical forces, such as covalent or hydrogen bonding, ensuring a robust connection between the bacterial cells and the surface (Petrova and Sauer, 2012). The irreversibility of this attachment is a key feature that distinguishes it from the initial, reversible phase. As bacteria become irreversibly attached, they begin to undergo phenotypic changes, initiating the expression of genes associated with biofilm formation. The bacteria start producing extracellular polymeric substances (EPS), which form a protective matrix around the cells. This matrix not only anchors the bacteria to the surface but also provides structural

integrity to the emerging biofilm. The irreversible attachment stage is a critical juncture in biofilm development, setting the foundation for subsequent events, including microcolony formation and maturation of the biofilm structure. Understanding this phase is essential for devising strategies to disrupt or prevent biofilm formation, addressing challenges in diverse fields such as medicine, industry, and environmental science (Arampatzi, *et al.* 2011).

***2.3. Formation of microcolonies or development*:** The third step in the mechanism of bacterial biofilm formation is the development of microcolonies. Following the irreversible attachment of bacteria to a surface, these attached cells start to proliferate and form small clusters known as microcolonies. This stage represents a pivotal transition in biofilm development, marking the beginning of the spatial organization of microbial cells. Microcolonies consist of densely packed bacterial cells encased within the extracellular polymeric substances (EPS) matrix that was initiated during the irreversible attachment phase (Guzmán, *et al.* 2021). The EPS matrix serves as a protective scaffold, providing structural support and allowing microcolonies to adhere more firmly to the substrate. Within these microcolonies, bacteria engage in intricate communication and cooperative behavior, facilitating the exchange of genetic material and metabolic cooperation. As the microcolonies grow and mature, they contribute to the three-dimensional architecture of the developing biofilm. This structural complexity enhances the resilience of the biofilm, making it more resistant to environmental stresses and external challenges (Zijnge, *et al.* 2010). The microcolonies continue to recruit additional bacterial cells from the surrounding environment, promoting the ongoing expansion and maturation of the biofilm. Understanding the formation of microcolonies is crucial for unraveling the dynamics of biofilm development and devising strategies to interfere with or disrupt these organized microbial communities. The complexity of interactions within microcolonies contributes to the robustness of biofilms and presents challenges in various fields, including healthcare, environmental science, and industrial processes (Karimi, *et al.* 2015).

***2.4. Maturation*:** The fourth step in the mechanism of bacterial biofilm formation is maturation. After the establishment of microcolonies, the biofilm enters a maturation phase characterized by the continued growth and development of the microbial community. During this stage, the biofilm structure becomes more organized and complex, with an intricate network of microcolonies embedded in the extracellular polymeric substances (EPS) matrix (Bowen, *et al.* 2018).

Maturation involves the recruitment of additional bacterial cells to the biofilm, contributing to its biomass. The EPS matrix plays a crucial role in providing structural integrity, allowing the biofilm to withstand various environmental stresses and challenges. This matrix also facilitates nutrient and waste exchange among the microbial cells within the biofilm. As the biofilm matures, it may develop channels or water channels that enhance fluid flow within the community. These channels support nutrient supply and waste removal, promoting the survival and persistence of the biofilm (Kostakioti, *et al.* 2013). The architecture of the biofilm becomes more heterogeneous, with distinct microenvironments that can harbor different bacterial species, contributing to the overall diversity of the microbial community. The maturation phase marks the biofilm's increased resistance to antimicrobial agents and host immune responses, making it more challenging to eradicate. This resistance is attributed to factors such as the protective EPS matrix, altered gene expression within the biofilm, and the formation of persister cells—dormant cells that are less susceptible to antimicrobial treatments. Disrupting the organized structure of mature biofilms is a critical aspect of addressing biofilm-related issues in various fields, including medicine, industry, and environmental management (Uruén, *et al.* 2020).

***2.5. Dispersal or detachment from the matrix*:** The fifth step in the mechanism of bacterial biofilm formation is dispersal or detachment from the matrix. After the maturation phase, biofilms undergo a dynamic process where some bacterial cells detach from the biofilm structure, becoming planktonic once again. This dispersal phase is essential for the life cycle of biofilms and influences their overall impact on host surfaces or environments. Biofilm dispersal can be triggered by various factors, including changes in environmental conditions, nutrient availability, or specific signalling mechanisms within the biofilm community. The goal of dispersal is to release individual or small groups of bacteria into the surrounding environment, allowing them to colonize new surfaces or contribute to microbial diversity (Kaplan, 2010). Several mechanisms facilitate biofilm dispersal. Enzymes produced by bacteria within the biofilm can degrade the extracellular polymeric substances (EPS) matrix, weakening the structural integrity and facilitating the release of bacterial cells. Additionally, the release of signalling molecules, such as autoinducers, can induce programmed dispersal, coordinating the departure of cells from the biofilm. Dispersed bacterial cells can then act as seeds for the formation of new biofilms on different surfaces. This dynamic process enables biofilms to adapt to changing environmental conditions and colonize diverse niches. However, the detached cells can also contribute to the spread of infections in medical settings (Rumbaugh and Sauer, 2020).

As soon as bacteria start to form biofilms, they attach to a single layer. Pretreatment of the surface is carried out using organic or inorganic macromolecules before joining. Numerous characteristics, including coarseness, permeability, hydrophobicity, hydrophilicity, and the surface's pore structure, are important factors that encourage bacterial attachment to any surface (Muhammad, *et al.* 2020) (Figure 1).

Numerous appendages, such as the flagella, pili, fimbriae, and glycocalyx, all have a substantial impact on the degree of attachment, allowing cells to maintain their attachment even in the presence of repulsive forces. The connected bacteria begin to proliferate constantly and establish microcolonies during the second stage (Yu, *et al.* 2017).

These microcolonies produce an extracellular polymer suite by employing quorum sensing. Through cell-to-cell contact, certain biofilm genes are expressed by tiny signalling molecules. As a result, the EPS matrix develops and forms the last stage. From the biofilm that has formed, bacteria scatter to begin a new cycle (Preda and Săndulescu, 2019).

All organic matter makes up between 50 and 90 percent of the content of EPS. Age and environmental factors may have an impact on the amount and make-up of EPS. When the cell mass is eliminated, cells separate from the biofilm, increasing the likelihood of infection by dispersing the infection inside the host and allowing detached cells from the biofilm to adhere to new places. The initial step of the microorganism's adhesion to the surface can be targeted during biofilm formation to stop the expansion of the biofilm. Inhibiting biofilm formation in its initial stage targets cell division and EPS synthesis by removing inactive cells, dissolving the EPS matrix, or altering the microenvironment. Physical removal of pre-formed biofilms is possible (Kim, *et al.* 2016) (Table 1).

3. Challenges in the Treatment of Oral Biofilm-Associated Infection

Oral antibiotics, while commonly used for treating periodontal issues, face challenges due to limited access to deeper areas, systemic side effects, and the emergence of microbial resistance. The shorter duration of action of oral antibiotics compared to topical medicines disrupts the natural microbial flora and contributes to resistance issues. The periodontal area's brief contact with oral antibiotics hinders their desired impact. To address this, periodontal dressing materials like PeriPas, SeptoPack, and Vocopac incorporate chlorthymol, silica, magnesium oxide, and synthetic glue, aiming to extend the interaction between the dosage form and the periodontal area (Kapoor, *et al.* 2012). Conventional medicines also pose problems such as microbial resistance and adverse effects (Waziri, *et al.* 2022). For instance, chlorhexidine can lead to a decrease in human keratinocytes and fibroblasts. Studies on Greek patients reveal a substantial percentage with periodontitis carrying bacteria resistant to Metronidazole and B-Lactum antibiotics. Tetracycline use has been linked to tooth discoloration. Traditional oral or topical treatments may struggle to effectively target the bacterial flora in deep periodontal pockets, necessitating innovative medicines to reduce side effects and enhance patient compliance (Soares, *et al.* 2012).

Dental biofilms are closely linked to serious conditions like apical inflammation, periodontal disease, and caries, significantly impacting dental health. The high resistance of oral biofilms to conventional antibacterial treatments poses challenges in managing related illnesses and infections. This resistance arises from universal biofilm mechanisms and the unique oral environment (Faveri, *et al.* 2006). The two main reasons for oral biofilm resistance are the shared mechanisms across all biofilms and the distinctive features of the oral cavity. Understanding these complexities is crucial for developing innovative medicines that overcome resistance challenges, minimize undesirable side effects, and ensure patient compliance. Novel approaches, such as extended-release periodontal dressings, hold promise in addressing these issues by providing sustained drug delivery to the target site, thereby enhancing the efficacy of periodontal treatments while minimizing systemic effects and microbial resistance (Ahmad, *et al.* 2022). Continued research and development in this direction are essential for advancing oral healthcare strategies and improving patient outcomes (Armitage, *et al.* 2010: Inui, *et al.* 2015).

3.1 Oral Biofilms and the Relations with Diseases

Some of the body's most intricate biofilms are found in the mouth. The distinctive and intricate nature of the oral environment is mostly to blame for this. According to studies, the human mouth contains up to 700 distinct kinds of microorganisms (Stewart and Costerton, 2001). Like the rich ecological habitats in tropical forests, the mouth is full of diverse and numerous microenvironments that offer multiple opportunities for the growth of a wide range of different microorganisms (Aslam, *et al.* 2021). Growth sites include smoother tooth surfaces, narrow and rough structures such as grooves on the surface of the tongue, wrinkles in the oral mucosa, fissures on the surface of the teeth, and gaps between the teeth. There are high-oxygen growth sites, as well as low-oxygen and anaerobic environments (Wang and Shao, 2017). What is more, the oral environment is in a constant state of dynamic change, which is susceptible to many external factors such as saliva, food, and oral hygiene habits, and the dynamic changes differ between individuals. Depending on where they colonize, oral biofilms can be categorized into different groups, including supragingival biofilm, also known as supragingival plaque, subgingival biofilm, endodontic biofilm, peri-implant biofilm, buccal mucosa biofilm, and tongue dorsum biofilm (Reynolds, *et al.* 2016).

Different colonization locations frequently have unique growth environments (different temperatures, oxygen tension, pH, and nutrition supplies, for example), as well as unique sensitivity to the host diet and dental hygiene practices (Pritchard, *et al.* 2017).

On the one hand, oral biofilms colonized in various locations have varying dominant flora and microecological compositions, which might result in varying mouth illnesses. For instance, a

supragingival biofilm on the tooth surface, a subgingival biofilm on the gums, and an endodontic biofilm on the tooth root could all result in periodontal disease (Liu, *et al.* 2017). On the other hand, oral biofilms's internal ecological and microbial composition are dynamic and vary as the related diseases develop and manifest themselves. That is, different multicell community effects displayed by the biofilm may correspond to different stages of the development of various biofilm-related disorders (Sheng, *et al.* 2023). For instance, in gingivitis, as the condition progresses from the beginning stage to the established stage, the predominant microbial species and subgingival biofilm in the gingival crevicular changes significantly: the proportion of anaerobic and facultative anaerobic bacteria gradually increases as the severity of the disease increases. (Besinis, *et al.* 2015).

3.2 Oral Biofilms and Their Relations with Drug Resistance

First, Extracellular polysaccharides (EPS), which surround microorganisms to provide a diffusion-modifying matrix, are abundant in a biofilm. As a result, the nonseparation surface's chemical and physical milieu within the biofilm membrane is significantly changed. The oral mucosa develops a biofilm structure, a persistent ecological colonization development
that modifies the physiological properties of the mucosa, including food metabolism (Ferrer and Mira, 2016).

Second, a biofilm is not merely a mass of oral bacteria that are free to grow. Opportunistic infections can persist in limited-nutrient zones where they may be dormant and resistant to antibiotics and antibacterial agents, in contrast to regular bacteria found in greater nutrient zones because a biofilm's micronutrient composition is structured in a gradient (Feres, *et al.* 2016). Third, in the ordered bacterial community of a biofilm there are symbiotic, reciprocal, and antagonistic relations among different bacteria species. The relationships between various bacterial species are not arbitrary; rather, they adhere to a set of laws (Wei, *et al.* 2019).

These laws are not set in stone and may adapt in response to changes in the environment, diet, and other contributing factors. Due to these modifications, biofilms at various growth phases exhibit varying sensitivity to anti-microbial treatments (Bowen, *et al.* 2018). When a bacterial species is present in a biofilm, its properties may change. For example, bacteria that are known not to have resistance genes may display resistance in biofilms, while less drug-resistant bacteria may display increased resistance in a biofilm environment. Additionally, horizontal gene transfer can lead to medication resistance in bacteria in biofilms (Patil, *et al.* 2008.

Due to these reasons, traditional explanations for the development of antibiotic transporters, the synthesis and modification of cell membranes, and drug efflux pumps are insufficient to explain the complex and variable drug resistance exhibited by bacterial populations in biofilms. Bacteria in biofilms may be using conventional resistance mechanisms, but these mechanisms may be more effective in this setting. It is widely accepted that complex interactions between the numerous bacterial populations living inside a biofilm are what cause the features of biofilms (Soto, 2013). This structure of the bacterial biofilm, known as the "multi-cell defense mode", is directly linked to the emergence and development of antimicrobial agent resistance. There are specific mechanisms of resistance that are intimately tied to the environment of the oral cavity in addition to the general resistance mechanisms outlined above that are present in most oral biofilms (Vestby, *et al.* 2020).

4. Physiological Factors of Bacterial Biofilms

4.1. pH- An infection may result in the creation of a microenvironment with high levels of acid due to metabolic activity or the response of our immune system. One species of bacteria that can withstand extremely acidic environments is Helicobacter pylori. Staphylococcus aureus (S aureus) is another instance, which produces organic acids during anaerobic fermentation processes (Yin, *et al.* 2021). As a result, H+ ions are produced and build up in the deeper layers of bacterial biofilms, which causes their pH to decrease and occasionally even approach 5.5! Because hazardous bacteria are now more resistant to antibiotics and thrive in low-pH conditions, the risk of secondary infections is rising (López, *et al.* 2010).

Antibiotics can kill the bacteria on the biofilm's surface, but their effectiveness is reduced by the acidic microenvironment. This condition would restrict the use of such medications, although they can increase the reactivity of polymeric nanoparticles (Khan, *et al.* 2021). To create pH-sensitive antibiofilm particles, two main strategies have been used, involving the placement of responsive groups at the shell or core of the particles, resulting in shell responding or core responding types, respectively (Guo, *et al.* 2021).

4.2. Enzymes- Proteases, lipases, and nitro reductases, for example, show a strong affinity for bacterial biofilms. P lactamases may be produced by such bacterial cells because of *in vivo* genetic changes, which may prevent peptide-based antibacterial medicines from working as effectively by hydrolyzing them until they are rendered inactive (Ramírez and Eckhard, (2022).

The antibiotics' failure to completely penetrate the biofilm renders their activity against bacteria within these films ineffective since the minimum inhibitory concentration (MIC) cannot be reached. When used properly, these specialized and selective enzymatic procedures enable the activation of polymeric nanoparticles that are responsive to enzymes (Lebeaux, *et al.* 2014).

4.3. Reactive Oxygen Species (ROS), Hypoxia, and other factors- Endotoxins can activate immune cells within their host, much like bacteria can. As a defence mechanism against prospective intruders, ROS is created in this situation (Moser, *et al.* 2021). When there is an imbalance between the amount of oxygen accessible and how it

is used in bacterial biofilms, hypoxia frequently results. When hypoxia is prevalent, it frequently leads to antibiotic resistance. Current research on polymeric particles sensitive to hypoxia-activated reactions and ROS is still restricted and incomplete when compared to nanoparticles that respond to pH or enzymes (Lee, *et al.* 2017, Aslam, *et al.* 2021).

5. Types of bacterial biofilms

Depending on how the surface and individual cells interact, biofilms can be monolayer or multilayer. Interactions between the constituent cells and the surface are more significant in a single-layered biofilm than interactions between the constituent cells themselves. Numerous different kinds of adhesive structures have been linked to the formation of the monolayer microbial biofilm (Gebreyohannes, *et al.* 2019). In one type of prepared adhesion structure, such as a flagellum or pilus, the formation of the monolayer biofilm is hastened, increasing transient attachments to the surface. A different form of microbial adhesin is produced concurrently with the change from temporary to permanent attachment (Haiko and Westerlund, 2013).

Microorganisms often create a multilayer biofilm by adhering to both surfaces and interacting with each other. Bacterial surfaces are known to cause repulsion under a variety of conditions. For example, the O antigen, which is normally negatively charged in nature, controls the chemistry of the cell wall of gram-negative bacteria (Sharma, *et al.* 2023). When creating a multilayer film, a similar charge between microorganisms creates an opposing force that must be neutralized (Khan, *et al.* 2022). It is possible to mask this negative by down regulating or mutating the genes that produce the O-antigen, adding divalent cations, creating extracellular polymeric substances (EPS), and other methods (Achinas, *et al.* 2019).

6. Current modalities for oral biofilm disruption-

Treatment and prevention of pathogenic oral biofilms are difficult. Topically applied formulations meant to stop the growth of biofilms have problems with rapid salivary elimination, poor EPS matrix penetration, and a lack of substantivity (i.e., retention on tooth surfaces). Since the presence of EPS and its altered microenvironment decrease drug availability and increase bacterial antibiotic tolerance, treating EPS without upsetting the normal microbial ecology is difficult (Benoit, *et al.* 2019). Additionally, many antibiotics are rendered useless by the acidic nature of tooth biofilms. Because oral biofilms are common and continuous, any therapy must be tolerated for a long time with little toxicity and side effects (Tian, *et al.* 2018).

Current treatments for confined oral biofilm control that use broad-range antimicrobial medications, such as chlorhexidine, are unsuitable for daily and long-term use due to their limited negative side effects, such as calculus formation and tooth discoloration (Ali, *et al.* 2024). Natural, on the other hand. Cariogenic biofilm development is inhibited and EPS synthesis is constrained by terpenoids, flavonoids, and essential oils with anti-biofilm characteristics (Rath, *et al.* 2021; Polizzi, *et al* 2022). These medications have an impact on S mutans' viability, acid generation, acid tolerance, and EPS synthesis at acidic pH values. Still, poor drug solubility, EPS diffusion, and substantivity impact these medicines' anti-biofilm action or efficacy (Rudin, *et al.* 2023).

7. **Nanomaterials based oral biofilm disruption-**

The difficulties in delivering medications to oral biofilm can be overcome by using nanoparticles. The flexibility of chemicals and simplicity of manufacturing of nanoparticles enable the creation of innovative biofilm disruption treatments (Kawish, *et al.* 2017). By using nanoparticles, bactericidal effects can be achieved without physical touch (Allaker and Yuan, 2019). They can also be designed to improve the solubility and penetration of medications into bacterial cells. Then, during the manufacture process, metals, metal oxides, natural synthesised polymers, or any mixture of them, must be used to give these TM particles anti-biofilm characteristics (Ramasamy and Lee, 2016).

Additionally, nanoparticles work with the mats to facilitate flexible and successful targeting and retention in oral biofilms. As a result, chemical compositions, surface charge, and size, among other qualities, are precisely adjusted to produce increased substantivity and anti-biofilm efficacy, which creates a synergistic effect (Jiang, *et al.* 2023). The intricate antimicrobial mechanism involved is capable of overcoming bacterial resistance mechanisms like multidrug efflux pumps or permeability regulation. In addition, these properties are adaptable enough to shield conventional medications from pH degradation, enzymatic attack, and microbial competition while lowering bacterial resistance to potent antibiotics. Because it provides for the specialised adaptation needed over time as a result of certain pathogenic stimuli like hypoxia or changes in PH levels, nanoparticle design is excellent due to its active response aspect (Hu, *et al.* 2019). Nanoparticles are utilised in root canal therapy and other restorative procedures to improve the mechanical strength of the dentin structure and alleviate hypersensitivity issues that might complicate tissue regeneration (Gao, *et al.* 2016; Imlay, 2013).

8. Classification of nanomaterial

Based on their composition, nanoparticles can be divided into two categories: those that are produced artificially or naturally. The actual particles themselves can be spherical, rod-shaped, tubular, or

Table 1. Types of oral biofilms and the associated oral diseases along with characteristics

Oral biofilm classification	Dominant flora	Associated oral diseases	Microecological characteristics	reference
Buccal mucosa biofilm	Streptococcus spp., Neisseriae veillonella	Oral mucosal recurrent aphthous stomatitis	Aerobic environment, Patients with oral mucosal disease have lower levels of the healthy core microbiota, such as Streptococcus salivarius, but higher levels of uncommon species, such as Acinetobacter johnsonii.	(Thomas, *et al.* 2021: Colombo and Tanner, 2019).
Endodontic biofilm	Obligate anaerobes (e.g., porphyromonas and prevotella)	Pulpitis, pulp necrosis, apical periodontitis	Difficult to totally eliminate or disinfect, Different clinical signs are associated with various prevalent microorganisms in an infected root canal.	(Narayanan and Vaishnavi, 2010)
Peri-implant biofilm	Gram+ aerobic, Gram+ facultative anaerobic bacteria	Peri- implantitis	Similar to subgingival plaque, in terms of structure, anaerobic bacteria such as P. gingivalis, T. forsythia, T. denticola, F. nucleatum, and P. intermedia greatly increased in number when there was inflammation.	(Kadkhoda, et *al.* 2016).
Subgingival biofilm	G+ cocci (e.g., S. sanguis and S. mitis), G+ bacillus (e.g., Actinomyces), G anaerobic bacteria (e.g., Spirochaetes)	root caries, gingivitis, periodontitis	The hypoxic stable environment which is rarely affected by se3lf cleaning, the propotion of anaerobic and facultative anaerobic bacteria gradually increases as the severity of diseases increases.	(Schulze, 2012).
Supragingival biofilm	streptococci spp. (e.g., S. mutans, S. sanguinis), facultative anaerobic spherical bacterium and anaerobic bacillus	Dental caries, gingivitis	G+ve bacteria are the predominant kind, an aerobic setting with a lot of oxygen, Significantly impacted by oral hygiene practises, Tendency is from Streptococcus to Lactobacilli in mature biofilms	(Inchingolo, et al. 2022: ; Nagata, et al., 2012).
Tongue dorsum biofilm	Streptococcus spp., Neisseriae Leptotrichia spp.	Halitosis, oral mucosal disease e.g., recurrent aphthous stomatitis	The tongue dorsum biofilm contained Treponema denticola, P. gingivalis, Tannerella forsythensis, Prevotella melaninogenica, P. intermedia, Fusobacterium spp., Streptococcus, and Actinomyces spp., accounting for around one-third of the oral cavity's bacterial population, Periodontal microorganisms may be able to infect or reinfect supragingival and subgingival plaque through this site.	(Bernardi, *et al.* 2020; Seerangaiyan, *et al.* 2017).

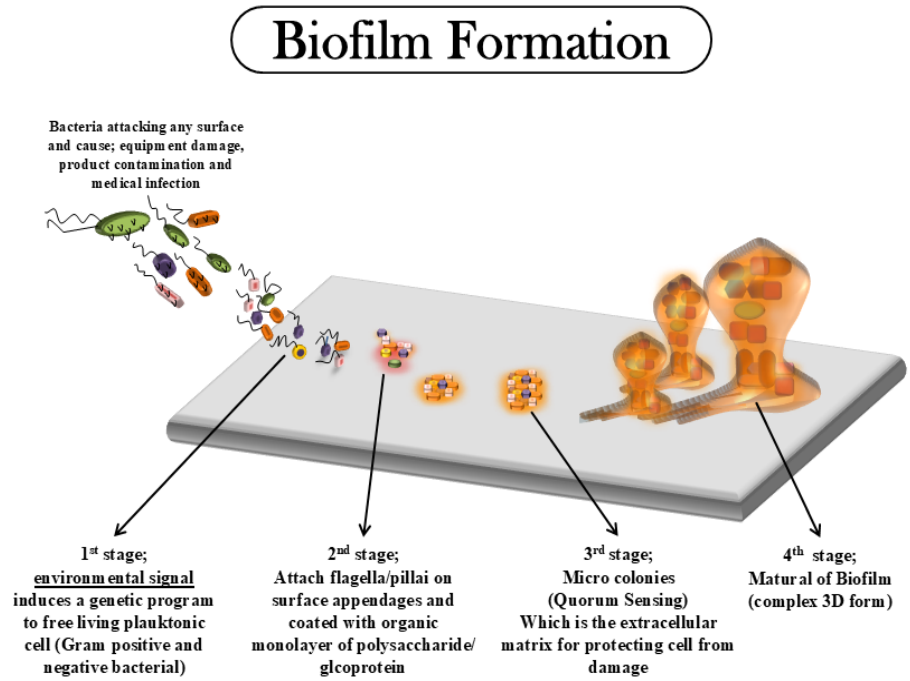

Figure 1. Different stages of Biofilm formation

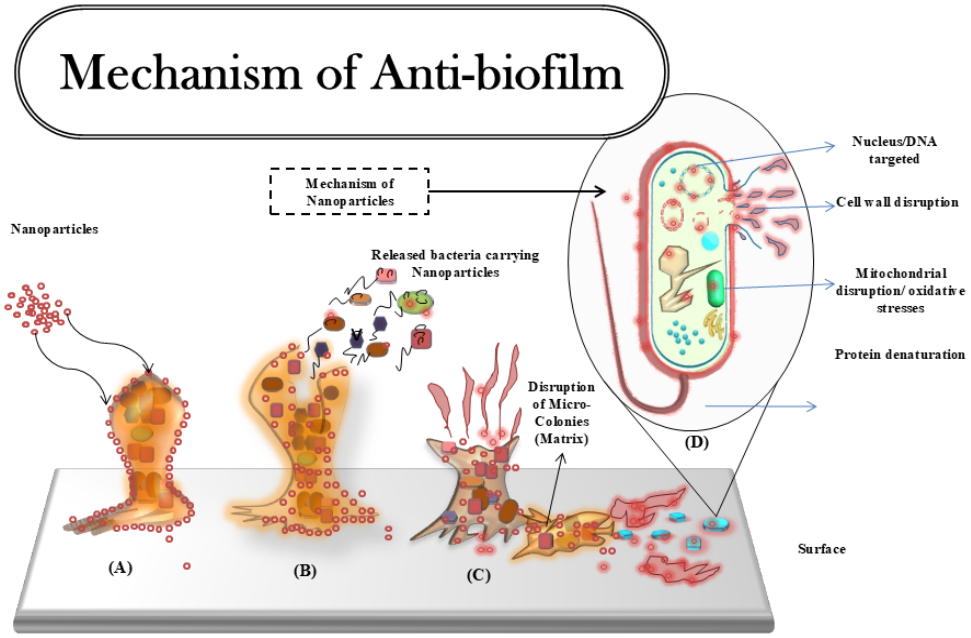

Figure 2. Diagrammatic representation of the mechanism of Anti-biofilm

plate-like in shape (Alam, *et al.* 2016). Additionally, functionalized nanoparticles can be identified by the presence of an inner core made of a single type of material that contains various molecules or by the presence of these materials' surfaces surrounding them (Harish, *et al.* 2022). Drugs, peptides, etc., which concentrate on working linker molecules with reactive groups at both ends capable of binding different molecule types (such as antibodies) onto cores composed of biocompatible materials like dextran and fluorophores being used where appropriate for detection purposes via imaging methods, may be needed to modify nanomaterials to suit specific applications (Fernández and Orozco, 2021). In the meanwhile, there are further ways to categorise nanoparticulate materials, such as sorting by dimensions, where fullerenes represent zero-dimensional objects and graphene is thought of as two-dimensional. These are all included in the category of composite nanomaterials, as it is often called (Saleh and Hassan, 2023) (Figure 2).

8.1 Graphene- A kind of carbon called graphene is incredibly thin, possesses a crystal lattice, and flows freely without any structural flaws. This substance aids in the creation of anti-bacterial surfaces as well as the demarcation and recognition of infections (Kumar, *et al.* 2019). Sodium hypochlorite is a one intracanal irrigant utilised for its microbial and tissue-dissolving characteristics. However, if extruded apically, it produces fast haemolysis and soft tissue ulceration, hence caution is advised while utilising this specific irrigation technique. The antibacterial properties of silver nanoparticles are maintained when graphene is added, but the cytotoxicity effects on bone and soft tissue are significantly reduced (Yared and Ramli, 2020).

8.2. Chitosan- Chitosan, which may be chemically altered, is the second most prevalent natural biopolymer after chitin. Its fundamental component is a deacetylated derivative. Its extraordinary capabilities are distinguished by its superior antibacterial and antifungal properties compared to other chemicals in this category, as well as its exceptional antiviral qualities. The mechanism used by Chitosan NP to kill bacteria effectively involves electrostatic interaction triggers that cause cell membrane breakdown, increase cellular wall permeability, and promote microleakage (Thambiliyagodage, *et al.* 2023).

8.3. Silver nanoparticles (AgNPs) - AgNPs have been widely used in dentistry, and there are now a wide variety of applications for them. Endodontics is one such area that is rising in prominence. A highly effective penetration into the bacterial cell walls is made possible by the small size and large surface area, which results in immediate bactericidal effects. Additionally, silver nanoparticles have positive biocompatibility traits and low levels of toxicity (Oncu, *et al.* 2021). While keeping their effectiveness for longer periods. When it comes to precisely treating Enterococcus faecalis through biological development including silver NPs, these features have shown a lot of promise. Despite its effectiveness, one must be aware of any potential downsides when employing application techniques, particularly in the case of anterior teeth, where discoloration may happen after applying AgNPs (Wu, *et al.* 2014).

8.4. Iron compound (FeOx) - Iron oxide (FeOx) nanoparticles are more relevant in the biological and medicinal domains (Rudin, *et al.* 2023). [90] Antibiotic- and immune-cell resistant exopolymers are produced, which makes it difficult to remove endodontic biofilms using them. Therefore, iron-based NPs are useful in completely getting rid of these germs. Dental implants can also be cleaned of biofilms using iron-oxide nanoparticles (Thukkaram, *et al.* 2014).

8.5. CuO nanoparticles (CuONPs) - CuO nanoparticles are a potent adversary for both gram-positive and gram-negative bacterial strains due to their capacity to permeate the bacterial membrane and interfere with their vital enzymes. They also have sonic antifungal properties. However, their use in endodontics is still limited, and more research is required to determine their efficacy or viability (Azam, *et al.* 2012).

8.6. Gold nanoparticles or nanorods- gold nanoparticles (AuNPs) have been found to be efficient against microbes through a variety of ways. The peroxidase, glucose oxidase, and superoxide dismutase enzymes, among others, are mimicked by the catalytic activity of Au nanoclusters and AuNPs. This characteristic, which resembles an enzyme, raises the formation of reactive oxygen species, which can put bacteria under oxidative stress (Sen, *et al.* 2020). Additionally, to this procedure. interacting with thiol groups on certain proteins, such as nicotinamide adenine dinucleotide (NADH), in an irreversible manner Dehydrogenases have also interfered with the reduction-oxidation equilibrium of bacterial respiratory chains, causing additional harm (Gao, *et al.* 2016).

Currently, a unique nano-formulation devoid of antibiotics and rich in Au nanorods has demonstrated notable effectiveness in treating pneumonia that is resistant to medication therapy. These gold jewels, which range in size from 50 to 100 nm, are covered in glycomimetic polymers that prevent the bacterial lectins needed for the development of bacterial biofilms from growing. It's interesting to note that when paired with lectin-blocking drugs, this novel substance effectively suppresses Pseudomonas aeruginosa infection by destroying of it by photothermal activity triggered by NIR light stimulation (Tang, *et al.* 2022).

9. Various Approaches for Delivery of Antimicrobial using Nanoparticles

Nanoparticles are useful drug carriers because of their small size in comparison to biological cells and unique physical and chemical properties, particularly in the case of antibiotics and anti-cancer medications that can target cells with specificity (Ahmad, *et al.* 2023). Due to the enormous challenges faced by the continual

proliferation of bacterial resistance, the need for novel and potent antimicrobial drugs has arisen. In terms of novel antimicrobial agents, metallic NPs seem to be the most promising because they have strong antibacterial properties. To serve as transporters or microbial defenses, respectively. Drugs that can boost effectiveness levels can be put into NPs (Edis, *et al.* 2021; Gavas, *et al,* 2021).

Without the use of medicines, a variety of ingredients, including metals, chitosan, and surfactants, are used to create antibacterial nanoparticles. Bacterial cell walls can attach positively charged cationic compounds to their negatively charged membrane surface, and certain NPS can rupture membranes because of their amphiphilic nature. When compared to conventional therapy, drug encapsulation into nanocarriers improves bacterial clearance and bioavailability by raising efficacy, fending off enzymatic attack while taking toxicity limits for drug release, or increasing half-life or bioavailability (Sánchez, *et al.* 2020).

While designing stimuli-responsive systems that recognize environments dynamically to facilitate targeted delivery using internal stimulants like enzyme concentrations linked with pathological conditions caused by infections along with inflammation changes in pH level, ligands present on bacterial surfaces help functionalize these carriers targeting specific pathogens for effective use (Rickard, *et al.* 2003; Deng and Liu, 2021).

10. Conclusion

In conclusion, the advent of nanoparticles represents a significant breakthrough in disrupting bacterial oral biofilms. The unique properties of nanoparticles, such as their small size and high surface area, enable them to penetrate and target bacterial biofilms more effectively than traditional antimicrobial agents. The ability of nanoparticles to interfere with the biofilm formation process, inhibit bacterial growth, and enhance the effectiveness of conventional oral hygiene measures has shown promising results in combating oral infections. The versatility of nanoparticles allows for tailored approaches in designing materials with specific antimicrobial properties, minimizing the risk of resistance development. This breakthrough opens new avenues for the development of advanced oral care products and therapeutic strategies that can address the challenges posed by persistent biofilms in oral health.

While the potential of nanoparticles in disrupting bacterial oral biofilms is evident, further research is needed to explore their long-term safety, potential side effects, and optimal delivery methods. Nonetheless, the current body of evidence suggests that nanoparticles hold great promise in revolutionizing oral healthcare by providing effective tools for preventing and treating oral infections associated with biofilm formation.

Author contributions

H.D. contributed to the conceptualization of the study and assisted with data collection. F.A. was responsible for the methodology design and data validation. F. contributed to data analysis and interpretation. K.Q. participated in drafting the manuscript and reviewing the literature. M.D. provided technical support and contributed to statistical analysis. P.J. reviewed the manuscript critically and provided constructive feedback. M.A. supervised the research and ensured its alignment with the objectives. M.S. coordinated the research activities, finalized the manuscript, and approved the final version for submission. All authors read and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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