Unveiling the Antibiofilm Arsenal: A Mini Review on Nanoparticles' Mechanisms and Efficacy in Biofilm Inhibition

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Abstract. Biofilms are complex microbial communities attached to surfaces, encapsulated in a protective extracellular matrix, leading to increased antibiotic resistance, and contributing to persistent infections and widespread diseases. This mini review aims to examine the antibiofilm potential of nanoparticles (NPs) such as graphene, zinc oxide, hydroxyapatite, silicon dioxide, sodium silicate, titanium oxide, silver, gold, palladium, and tungsten. These NPs employ various mechanisms to disrupt biofilms, including sharp nanosheet attacks on cell membranes, alteration of mRNA expression, generation of reactive oxygen species that cause oxidative damage to essential biomolecules, and destabilization of the biofilm matrix. These multifaceted mechanisms underscore the potential of NPs in combating biofilms and addressing antibiotic resistance, offering promising avenues for future medical, industrial, and environmental applications.

Keywords: Antibiofilm, Antimicrobial, Biofilms, Nanoparticles, Nanotechnology.

1.0 Introduction

Biofilms are structured communities of microorganisms that adhere to surfaces and are embedded in a self-produced extracellular polymeric substance (EPS) [1,2]. These biofilms can form on various surfaces, including medical devices, industrial pipelines, and natural environments [3]. Biofilms are notoriously resistant to antimicrobial agents and immune responses due to their complex structure and protective EPS matrix, posing significant challenges in healthcare and industry [4]. Combating biofilm infections requires a multifaceted approach, leveraging antibiotics, antifungals, disinfectants, nanoparticles, combined antimicrobials and natural products [5-10]. Antibiotics remain a frontline defence, although their efficacy is often hampered by the biofilm matrix which protects bacteria and facilitates resistance development. Figure 1 summarizes the biofilm life cycle. The biofilm life cycle consists of several stages. It begins with the initial attachment of free-floating (planktonic) bacteria to a surface, where they adhere

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reversibly [11]. Following this, the bacteria enter the microcolony formation stage, clustering together and initiating communication through quorum sensing [12]. As the biofilm matures, it progresses to the biofilm formation (maturation) stage, where the bacterial cells are encapsulated in an extracellular polymeric substance (EPS) matrix that provides structural support and protection. This stage includes the development of water channels for nutrient and waste exchange [13]. Finally, during the dispersion stage, cells are released from the biofilm, returning to their planktonic state to colonize new surfaces [14]. This dispersal phase is essential for the propagation and survival of the bacterial community, enabling the spread of biofilm to new environments.

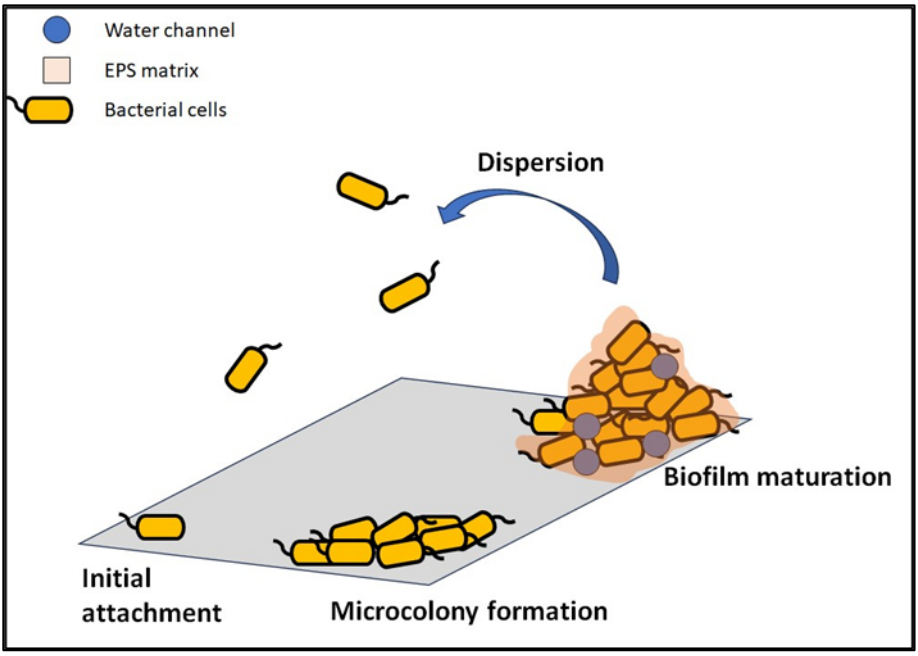

Fig. 1. Biofilm life cycle.

The utilisation of nanoparticles (NPs) is a highly promising approach to combat microbial resistance. NPs are particles with dimensions less than 100 nanometers. They exhibit unique physicochemical properties, such as high surface area-to-volume ratio, quantum effects, and enhanced reactivity [15, 16]. These properties make nanoparticles effective antimicrobial agents, particularly in disrupting biofilm formation and eradicating existing biofilms [17]. This mini review discusses the antibiofilm potential of various nanoparticles namely graphene, zinc oxide, hydroxyapatite, silicon dioxide, sodium silicate, titanium oxide, silver, gold, palladium, and tungsten.

1.1 Graphene

Graphene is a single layer of carbon atoms arranged in a hexagonal lattice, providing it with remarkable properties. It exhibits extraordinary electrical and thermal conductivity, mechanical strength, and flexibility. Its atomic thickness is about 0.345 nm, making it one of the thinnest yet strongest materials known. The sharp edges of graphene-based materials can physically damage bacterial cell membranes, leading to cell death and biofilm disruption [18]. Graphene oxide generates reactive oxygen species (ROS), causing oxidative stress and damaging cellular components like DNA, proteins, and lipids [19]. Graphene nanocomposites with metal nanoparticles release metal ions, enhancing antimicrobial efficacy by disrupting microbial metabolism and biofilm structure. In addition, He et al. [20] revealed that seven matrix-assisted laser desorption ionization time-of-flight (MALDI/TOF)-identified proteins were found induced (including chain A of the outer membrane enzyme Pagl, twitching motility protein PilH, ribosome recycling factor and arginine/ornithine binding protein AotJ) and nine proteins were suppressed (including amidotransferase, 30S ribosomal protein S6, arginyl-tRNA synthetase, and succinate dehydrogenase flavoprotein) in *Pseudomonas aeruginosa* by graphene-based silver nanoparticles (Ag NPs–GE).

1.2 Gold (Au)

Gold (Au) has an FCC crystal structure and is highly valued for its malleability, electrical conductivity, and resistance to corrosion. Gold nanoparticles, typically ranging from 1 to 100 nm, find use in medical diagnostics, electronics, and as catalysts. Upon exposure to specific wavelengths of light, gold nanoparticles generate localized heat, disrupting biofilm structure and killing bacteria [21]. According to Badoni and Prakash [22], gold nanoparticles induce the creation of ROS within biofilm cells, which leads to protein aggregation and DNA oxidation. A transcriptomic study revealed that gold nanoparticles killed Gram-negative bacteria via differential expression of genes associated with cell wall biosynthesis, glycolysis, TCA cycle, oxidative phosphorylation and DNA replication [23].

1.3 Zinc Oxide (ZnO)

Zinc oxide (ZnO) has a hexagonal wurtzite crystal structure and is recognized for its high thermal and chemical stability, semiconductor properties, and ability to protect against UV radiation. ZnO nanoparticles typically range from 20 to 100 nm in size, making them useful in various industrial and cosmetic applications. Zinc oxide nanoparticles are known for their antimicrobial properties against a broad spectrum of microorganisms, including biofilm-forming bacteria. ZnO nanoparticles produce ROS under light irradiation, contributing to oxidative stress in bacterial cells [24]. The dissolution of ZnO nanoparticles releases zinc ions, which interfere with microbial enzyme systems and membrane integrity, leading to cell death [25]. Furthermore, Abdelraheem et al. [26] revealed that ZnO significantly downregulated the expression level of virulence genes in *P. aeruginosa* biofilm namely LasR, rhlI, pqsR, LecA, PelA, exoS and lasA.

1.4 Hydroxyapatite (HAp)

Hydroxyapatite, which mimics the mineral component of bones, has a hexagonal crystal structure. It is biocompatible and bioactive, making it suitable for medical implants and bone grafts. Hydroxyapatite nanoparticles typically range from 20 to 100 nm in size. Hydroxyapatite nanoparticles release calcium ions, which can disrupt biofilm matrix and inhibit microbial adhesion [27]. Hydroxyapatite surfaces can adsorb antimicrobial agents, enhancing their local concentration and effectiveness. Huang et al. [28] demonstrated that hydroxyapatite reduced the relative mRNA expression of 16S rRNA, polB, cyd, GAPDH, and mdoG in *Escherichia coli*.

1.5 Silicon Dioxide (SiO_2)

Silicon dioxide (SiO_2), or silica, features a tetrahedral network where each silicon atom is bonded to four oxygen atoms. This arrangement gives it hardness, chemical inertness, and transparency to visible light. SiO_2 nanoparticles usually range from 10 to 100 nm and are widely used in glass, electronics, and food products. SiO_2 is known to disrupt fungal cell membranes, leading to a change in the membrane permeability and abnormal diffusion of ions [29]. Muslim et al. [30] reported that SiO_2-conjugated lectin significantly ($p < 0.05$) inhibited biofilm formation by reducing expression of rhlR gene.

1.6 Sodium Silicate (Na_2SiO_3)

Sodium silicate, often known as water glass, is an amorphous or glassy material composed of various silicate anions. It typically has an average size of 30 nm. It is also water-soluble and is used as an adhesive, in detergents, and in water treatment processes. Sodium silicate generally exists in solution form rather than as discrete particles. Sodium silicate creates an alkaline environment, which can inhibit microbial growth and disrupt biofilm structure [31]. On the other hand, silicate nanocomposite interferes with the mid layer and upper layers of the biofilm matrix [32].

1.7 Titanium Oxide (TiO_2)

Titanium oxide (TiO_2) typically occurs in three main crystalline forms: anatase, rutile, and brookite, each with distinct structural characteristics. It is valued for its high refractive index, strong UV light absorption, and photocatalytic properties. Titanium oxide nanoparticles are commonly sized between 1 to 100 nm, and they are extensively used in applications ranging from sunscreens to catalysts. TiO_2 generates ROS, including hydroxyl radicals and superoxide anions, upon UV irradiation, which degrade the biofilm matrix and kill embedded bacteria [24]. TiO_2 nanoparticles adhere to bacterial cells, causing cell wall damage and subsequent cell death through direct contact [33].

1.8 Silver (Ag)

Silver (Ag) has a face-centered cubic (FCC) crystal structure and is well-known for its high electrical and thermal conductivity, as well as its antimicrobial properties. Silver nanoparticles range from 1 to 100 nm in size and are used in medical devices, electronics, and antimicrobial coatings. Silver nanoparticles release silver ions (Ag^+), which bind to microbial proteins and DNA, disrupting cellular functions and biofilm formation [18]. Silver ions catalyze the production of ROS, leading to oxidative damage in biofilm-associated bacteria. Silver nanoparticles interact with bacterial cell membranes, increasing permeability and causing cell lysis [25].

1.9 Palladium (Pd)

Palladium (Pd) also has an FCC crystal structure and is noted for its excellent catalytic properties, particularly in hydrogenation reactions, and its high absorption of hydrogen. Palladium nanoparticles range from 1 to 100 nm and are utilized in catalytic converters, electronics, and hydrogen storage applications. Palladium nanoparticles catalyze the production of ROS leading to oxidative stress [34] which may cause biofilm disruption. Palladium nanoparticles can inactivate cellular enzymes and interact with bacterial membranes, causing structural damage and cell death [35].

1.10 Tungsten (W)

Tungsten (W) possesses a body-centered cubic (BCC) crystal structure, known for its high melting point, hardness, and density. Tungsten nanoparticles, ranging from 1 to 100 nm, are used in electronics, high-temperature applications, and as catalysts. Tungsten nanoparticles catalyze the production of ROS, leading to oxidative stress and biofilm degradation. Tungsten nanoparticles interact with bacterial cell membranes, increasing permeability and causing cell lysis [35, 36].

1.11 Summary of Antibiofilm Mode of Action

Figure 2 summarizes the potential antibiofilm mode of action by various nanoparticles. Nanoparticles combat biofilms through several mechanisms. Oxidative stress is induced by reactive oxygen species (ROS) generated by nanoparticles, leading to cellular damage and apoptosis [38]. This stress causes damage to biomolecules such as single stranded mRNA, double stranded DNA, enzymes, and lipids, disrupting essential cellular processes and leading to cell death [39]. The sharp nanosheets attack mechanism involves the physical piercing of bacterial cell membranes by the sharp edges of nanomaterials like graphene oxide, causing leakage of cellular contents and subsequent cell death [40]. Additionally, photocatalytic activation occurs when nanoparticles are exposed to light, enhancing their antimicrobial activity by producing more ROS and facilitating photothermal destruction of biofilm matrix [22].

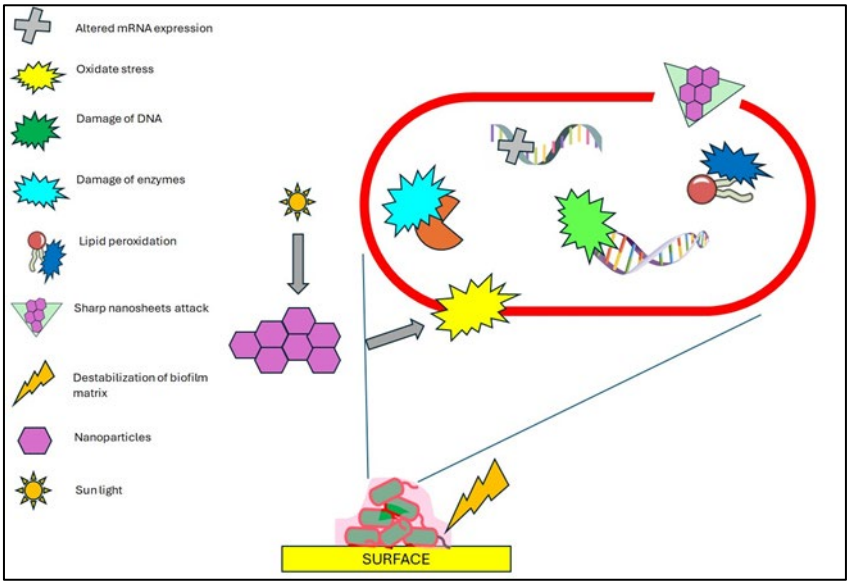

Fig. 2. Antibiofilm mode of action of nanoparticles.

2.0 Conclusion

Nanoparticles offer a promising solution to the persistent challenge of biofilm-associated infections. Their unique mechanisms of action, including physical disruption, ROS generation, and ion release, provide multiple pathways to combat biofilms. Future research should focus on optimizing nanoparticle formulations and understanding their long-term effects and safety profiles to ensure their effective and sustainable application in various fields.

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