

TARGETING MICROBIAL BIOFILMS ALTERING CHRONIC WOUND HEALING: NEW BREAKTHROUGH IN DRUG DEVELOPMENT USING SILVER

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DOI: 10.47750/pnr.2022.13.506.264

Abstract

Biofilm development is a potent inducer for many microorganisms that inflict chronic infections. Conventional antimicrobials face significant hurdles in dealing with biofilms and drug resistance because of the multi-factor nature of these processes. Despite this, bacterial biofilms remain a mystery, and their management methods are still lacking. Biofilms may lead to persistent illness and need surgical excision of affected regions if standard antimicrobial treatments fail to eliminate them. One of the most significant concerns in antibacterial medication research is discovering effective treatments for biofilm infections. Recent wound biofilm infection therapy research focuses on nanotechnology-based medication delivery systems and combination therapies. Biofilm-targeting medications are still in the early stages of research, although several promising techniques are already being tested. Current understanding of biofilm physiology and pathology is reviewed and discussed here, as is how a thorough understanding of biofilm physical and biological properties might influence treatment techniques and molecular targets for the creation of anti-biofilm medications. Specifically, we are interested in treatment approaches targeting the extracellular matrix, dormant cells, additional crucial microbial biofilm structure, functional features, and drug tolerance mechanisms. In many clinical investigations, silver has been found to improve the healing of chronic, non-healing wounds, including silver nanoparticles. The purpose of this review is to provide an overview of recent developments in silver nanoparticle-based biofilm therapeutics and to understand the current development path.

KEYWORDS: Microbial Biofilms, Chronic Wound Healing, Extracellular Matrix, Silver Nanoparticles.

INTRODUCTION

Biofilms are characterized as adherent bacteria to surfaces, encased in a self-produced extracellular matrix, and resistant to antimicrobial treatments (including antibiotics and anti-microbials). Furthermore, biofilm production is sometimes defined as a three- to five-stage process that begins with single cells adhering to a surface, then progresses to biofilm maturation and, finally, bacterial dissemination from the biofilm [1-3].

Multi-omic and imaging technologies have shown incredible intricacy and spatial structure [5]. Microbial biofilm development is a significant virulence factor in many localized chronic disorders, despite its first description as an obscure bacterial population behaviour. Infections caused by biofilms might recur even after a lengthy absence from the patient's care. While genetic mutations are not solely responsible for this phenomenon, increased cell density might enhance the spread of resistance genes. On the other side, biofilm bacteria may also

acquire antibiotic resistance via metabolic dormancy or molecular persistence programmes. Furthermore, the extracellular matrix's critical function in providing biofilm antimicrobial resistance is becoming identified [4].

Even though cell signalling's 'universal' Role in biofilm creation was recognized 20 years ago, signalling-based treatments for biofilm-associated infections have yet to be introduced due to cell signalling networks' complexity. In a similar vein, the development of materials science and surface modifications incorporating adhesion-targeting technologies, as well as surface textures or biomimicry and chemistries from plants and animals, were promising approaches to preventing microbial adhesion and subsequent biofilm formation [6].

Researchers have recently begun focusing on the extracellular polymeric substance (EPS) matrix as a potential attack site. In contrast, the variety of EPS matrix components and their interactions offer varying degrees of complexity and difficulty in developing EPS-targeting therapeutics [7,8].

Since penicillin and other antibiotics were found, silver and other non-antibiotic therapies were abandoned, but silver has garnered significant attention due to the advent of antibiotic-resistant strains and its low potential to acquire resistance [9]. Because of their inherent therapeutic qualities and multi-site action, silver nanoparticles have broad-spectrum antibacterial power against various microorganisms. They have a significant potential to address developing difficulties in microbial resistance in various applications [10-12].

Silver has been proven in vitro to be effective against biofilms. Moreover, there is substantial clinical evidence supporting the use of silver Nanotechnology in wound treatment. [13,14]

With an emphasis on silver's involvement in targeting microbial biofilm during the healing process of chronic wounds, this review hopes to provide the reader with a down summary of the latest advancement in silver nanotechnology.

THE CHARACTERISTICS OF MICROBIAL BIOFILM:

The extracellular polymeric substance (EPS) that helps bacteria adhere to a suitable surface forms biofilms, dense communities of bacterial populations [15]. Although numerous species may create wound biofilm, the two most prevalent bacteria responsible for wound biofilm, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, have received the most attention. Because it is unethical to develop biofilms in humans, most current understanding comes from in vitro and animal studies [16]. A meta-analysis of published data shows that biofilms are present in 78.2 per cent of chronic wounds, posing a considerable hazard to healing [17]. Biofilm bacteria are active and aerobic and proliferate rapidly in cultures, especially in the outer layers of the biofilm. If you are in a low-oxygen or anaerobic environment, deep-seated bacteria may be able to live for long periods and grow poorly in distinctive cultures [18,19]. Substantial genetic modifications modify their phenotype, resulting in inadequate identification by the innate immune system's Toll-like receptors (TLR)[20]. The EPS also acts as a physical barrier against immunological assault and medication interactions [21,22]. Residents interact with one another using quorum sensing (QS) molecules. Some also function as bacterial virulence factors, shielding the colony from immunological attack. A biofilm is likely to be present in a chronic wound with red, friable granulation tissue coated by a slimy coating that returns after debridement, increasing exudate production and indications of a retreating epithelial margin [23]. It might be related to slough, dead necrotic tissue at the wound bed [24]. Biofilm is an independent component that causes delayed wound healing [25].

BIOFILM FORMATION

It is possible to divide the formation of biofilms into five distinct stages: Attachment, Growth, Proliferation, Maturation and Dispersion

Attachment:

Because of the minerals in hard water, a layer of proteins and carbohydrates is only weakly connected. Because of this, microbial cells are attracted to the surface and cling to it. Bacteria must connect to a suitable surface for biofilm development to occur. A rough or textured surface and the desired conditioning coating are common characteristics of this material, often used in solid-liquid interfaces [26-30]. The attachment has been demonstrated to be affected by various parameters, including hydrophobicity, polarity, pH, hydrodynamics, and temperature. Free-floating bacteria use structures like fimbriae, pili and flagella to cling to surfaces [31-33]

Growth/ Irreversible attachment

The attraction forces between cells are more vital; therefore, they are more difficult to remove. This marks the beginning of maturation, an irreversible step [34,35].

Proliferation:

By releasing EPS (an extracellular polymeric material), bacteria attach themselves to the surface and each other, forming a matrix with a glue-like consistency.

Maturation:

Bacteria thrive in the biofilm environment's nutrient-rich layer. Oxygen, nutrients, and other growth-supporting materials are transported throughout a mature biofilm through several complicated diffusion channels, eliminating waste and dead cells [36,37].

Dispersion:

Cells actively developing lose their daughter cells over time, allowing the biofilm to disperse [38]. This is because as long as new nutrients are available, biofilms continue to proliferate, and when they are deprived of nutrients, by removing themselves from the water's surface, they return to a planktonic state. Bacterial cells may receive enough nutrients via this method [39]. All two varieties of *Vibrio* reconquer the interface after about five hours, suggesting that the detachment process could be species-specific. *Vibrio parahaemolyticus* recolonizes after roughly four hours [40].

Internal layers of cells are protected from spreading by EPS, which keeps them attached to the surface, whereas outer layers may separate from the colonies and spread to other parts of the host, culminating in systemic infections and even acute events like embolisms (a ruptured blood vessel) [41]. Due to poor nutrition and trash buildup, individual cells and clusters of cells are pushed to the film's periphery.

THE BIOFILM'S COMPOSITION:

There seem to be many microorganisms in the biofilm, but they are not randomly dispersed in the glycocalyx. The majority of the time, these microcolonies take the form of rods or mushrooms; however, they may include a variety of microorganisms. By volume, 10–25 per cent of microbial cells are found in microcolonies, which account for 79–90 per cent of the total matrix [42,43]. An abundance of EPS discharge might be seen as a sign that bacterial growth is abundant. The polysaccharide matrix holds together microorganisms at the bottom of many biofilms with other organic and inorganic components. The following layer, which is irregular and loose, can extend into its surroundings [42].

Aqueous media:

A primitive circulatory system exists between micro-colonies, exchanging nutrients and removing harmful compounds [43].

Exopolysaccharide (EPS):

The organic matter inside a biofilm accounts for between 50 and 90 per cent of its total mass. The main component is an exopolysaccharide (EPS), a carbohydrate generated by bacteria. This bacterium's primary component is polysaccharides, which may be cationic in Gram-positive bacteria, while others are neutral or polyanionic in those microorganisms. Anionic polysaccharides may be identified by the presence of uronic acids (such as D-galacturonic, mannuronic, and D-glucuronic acids) or ketal-linked pyruvate. When magnesium and calcium are cross-linked with polymer strands, they provide a more powerful binding effect in established biofilms. This anionic characteristic is essential in joining divalent cations like magnesium and calcium [44]. In addition to polysaccharides (1–2% of EPS), DNA (1%), proteins (1–2% (including enzymes), and RNA (1%), as well as particular lipids and humic chemicals, EPS contains these components [45].

eDNA:

Bacterial genetics and the environment in which they grow define biofilm structure. eDNA was first discovered in *Streptococcus intermedius*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus*. Autolysis is a systematic process for the release of eDNA. Released eDNA plays a crucial role in biofilm growth, biofilm structure stability, and gene transfer mechanisms. As a result of medical treatment selective pressures, circulating strains acquire virulence and antibiotic resistance genes through this genetic transfer. Examples of this include *Streptococcus pneumoniae* and other *Streptococci* [46].

BIOFILM AND DRUG RESISTANCE:

Biofilms are far more resistant to antimicrobial treatments than bacteria in a planktonic culture because they are created. A 600-fold increase in sodium hypochlorite (an oxidizing biocide used in most successful antibacterial treatments) has been demonstrated to be required to kill *Staphylococcus aureus* biofilm cells, for example [47]. A biofilm's unique physiology, such as its slower metabolic rate and better cell-to-cell communication, makes it

easier for bacteria to build resistance to antibiotics or lessen antibiotics' effects than planktonic bacteria [48]. Three assumptions have been made to characterize antibiotic resistance in biofilms:

1. Antibiotics diffuse slowly or partially into the innermost layer of biofilms. Since the biofilm-encrusted bacteria inside the EPS matrix provide a barrier to diffusion, this is why [43].
2. Some biofilm-dwelling microorganisms face developmental delays or famine due to food shortages or toxic metabolite accumulation. Many antibacterial medications are ineffective in treating these microorganisms [49].
3. The process of bacterial subpopulation differentiation is similar to spore generation. It has a specific and highly resistant phenotypic shield for its antibacterial actions [43].

Additionally, the biofilm's neutralizing enzymes contribute to antibiotic resistance. Hydrolysis and antimicrobial modification are two metabolic mechanisms these proteinaceous enzymes use to break down or inactivate antibiotics [45]. Although intense and persistent antibiotic therapy is beneficial in lowering biofilm and managing exacerbations of chronic biofilm infections, *In vivo* attainment of the minimum antibiotic concentration (necessary to eliminate a mature biofilm) could be difficult. Consequently, getting rid of an illness caused by a bacterial biofilm is difficult once it has grown. Antibiotic treatment alone is ineffective in eliminating biofilm infections in most cases in experimental trials [50]. In one study, *P. aeruginosa* biofilms were exposed to ciprofloxacin-loaded poly nanoparticles functionalized with DNase I. The extracellular DNA that stabilizes the EPS is the key to targeting and dismantling the biofilm [48]. They also release ciprofloxacin in a regulated way. Biofilm combination treatment is commonly advised for treating biofilm infections since it is much more effective than antibiotic monotherapy [50].

THE SIGNIFICANCE OF BIOFILMS IN THE DELAYED CHRONIC WOUNDS

Increasing antimicrobial and multidrug resistance levels is now a prominent discussion issue worldwide. Single-celled microorganisms are often sensitive to antibiotics unless they are fundamentally resistant. The susceptibility patterns provided by the clinical microbiology laboratory are used by most doctors engaged in the treatment of wounds to decide which antibiotics a patient needs. International consensus recommendations, which are adequate for treating acute infections [51-54], frequently assist in making these judgments. These recommendations may fall short of your needs for chronic conditions like cystic fibrosis (CF), lung infection, or a persistent wound that will not heal. Biofilms and planktonic are two separate phenotypic growth forms that bacteria may take on simultaneously. As a result, all classic microbiology and the development of antimicrobial agents were entirely based on planktonic concepts discovered in the early 1800s. For showing the pathogens of acute infections, these approaches are still universally considered the "gold standard." using shaken cultures or spreading on an agar plate, it is much simpler to grow bacteria, which is likely how bacteria live during acute illnesses. It is a very different storey with long-term infections. To defend themselves from being destroyed by neutrophils and macrophages, the bacteria in the biofilms are encased in a thick matrix of polysaccharides and free DNA (eDNA) from either the bacteria or the host as binding proteins that are securely attached to biofilm structures. Various bacterial species are present in most chronically infected wounds, necessitating several antibiotics [55-57]. However, multiple species are not always found in the same biofilm but rather in tiny, autonomous islands of a single species [58-60]. Several other factors contribute to antibiotic resistance, including that many bacteria are not proliferating fast or metabolizing quickly, making them more resistant to antibiotics.

NANOTECHNOLOGY:

Another rationale for seeking attention in nanotechnology, especially nanoparticles and nanorods, is its adaptability as a therapeutic and diagnostic tool. Challenges such as poor antibiotic penetration and non-targeted methods of distribution in biofilm-associated infections may be alleviated using nanotechnology. Biofilm research may be broken down into studies on the use of nanoparticles alone or combined with other treatments [61,62]. NP-based treatments for biofilm-associated illnesses are feasible because of their particular chemical and physical features; the colossal surface area to volume ratio enables them. Different NPs have been shown to have anti-biofilm properties, which are currently being studied. Many parameters, including the NP type, bacterial strain, NP concentration, quantitative methodologies, and the biofilm growth environment, must be considered to accurately define NPs' ability to disrupt or reduce biofilms. An overview of current studies on the production, characterization, and assessment of NPs to prevent or treat biofilm infections is presented here [63]. Biofilm infections may be prevented, disrupted or dispersed using different nanoparticle therapies. In order to

ensure appropriate dosage within the treatment regimen at the location of interest, NPs enable drug delivery with pre-determined kinetics [65].

Encapsulation or surface attachment of medicinal substances is possible, depending on the kinetics and chemical composition of the design. Gentamicin, for example, has concentration-dependent effectiveness; in other words, the medication released by the Nanoparticles may quickly and effectively eliminate all germs with a single burst. Because the antibacterial activity of beta-lactam depends on extended exposure, continuous release of beta-lactam is essential [64].

Because of the potential for improved bioavailability and targetability, the advantages of nanoparticles are heavily reliant on the delivery method [67]. A variety of local and systemic administration options are available for the delivery of NPs. These include wound dressing administration, local injection and aerosol. NPs has been employed to convey encapsulated medications beyond the mucus barrier in the lungs to deliver therapeutic molecules to the lungs of CF patients [66]. This is a step up from exhaling free drugs that had no impact. Many biofilm-targeted NP treatments have been adopted from the cancer therapy sector because of the richness of information in the usage of NPs. Increased vascular permeability is a common characteristic of cancer and infection-induced inflammation that determines the efficiency against biofilm infections of NP carriers designed for prolonged circulation and passive targeting in cancer treatments [68,70]. However, the primary differences between biofilm infections and malignancies cannot be ignored, and the specific properties of biofilms, throughout the NP design process, such as the decreased pH at the infected site [69].

SILVER NANOPARTICLES

Silver compounds have been used to disinfect wounds since antiquity [71]. However, the age of antibiotics started with the discovery of penicillin, and silver was mostly forgotten. Silver nanoparticles have emerged as a new area of research because of the rising concern about antibiotic resistance in bacterial infections and biofilms. One to 100 nanometers in diameter, silver NPs typically contain 20 to 15,000 silver atoms [73]. Additionally, silver nanoparticles are a broad-spectrum antimicrobial that kills Gram-positive bacteria and Gram-negative bacteria, along with fungus and viruses, due to their antibacterial properties [72]. There are many ways to make silver nanoparticles. To prevent silver NPs from aggregating, the most typical chemical approach converts Ag^+ (AgNO_3) is reduced to an Ag ion in the presence of a stabilizer, including such polyethene Glycol utilizing sodium borohydride as a reducing agent (N-vinyl-2pyrrolidone). Particle integrity may be improved by carefully selecting reducing and stabilizing agents [74]. Silver NPs may be produced in more environmentally friendly ways using live organisms like bacteria. When *Bacillus licheniformis* is mixed with an AgNO_3 solution in a flask and shaken for 24 hours at 37°C, particles are formed. Silver nanoparticles are extracted from cells and purified [74]. A biopolymer, like starch, may be used as a stabilizer in the production of silver NPs with little environmental impact [75]. For up to 90 days, silver nanoparticles in corn starch solution were shown to be colloidally stable. After 48 and 24 hours of treatment, *Pseudomonas aeruginosa* biofilm reduction was detected at 88% and 85%, respectively, demonstrating an efficient resistance of biofilm development with these starch-stabilized particles [75].

Citric acid-capped silver nanoparticles from 10 to 100 nanometers in diameter, aztreonam, and a combination of the two were used to cure biofilms of *Proteus aureus* (*P. aeruginosa*). Particle size was critical before applying silver NP treatments to prefabricated biofilm. According to Habash and colleagues, biofilm biomass and viability were reduced more effectively by silver nanoparticles (10–20 nanometers) than 100 nanometers. However, neither the biofilm structure nor the cellular morphology changed [76]. For planktonic bacteria, 8.3-nanometer silver NPs showed little effect, but when injected at a dose of 100–150 grammes per litre (g/ml), they killed most of the biofilm bacteria in *E. coli* biofilms. Another study [77] found that silver nanoparticles coated with Chitosan did not disrupt 24-hour-old biofilms of *P.aeruginosa* or *Staphylococcus aureus* but did considerably inhibit the growth of *P. aeruginosa* nanoparticles. Palanisamy et al. [78] found that chemically generated silver NPs (20 nm–30 nm) had a similar effect on *P. aeruginosa* biofilm inhibition.

Antibiotics and silver nanoparticles have been used in several experiments by researchers. Aztreonam alone failed to eradicate biofilms in one study but instead stimulated the production of new biofilms, up to a quarter of the untreated biomass, when treatment was discontinued[76]. CF patients with lung infections caused by the multidrug-resistant bacterium *P. aeruginosa* may benefit significantly from cationic antimicrobial peptides or CAMPs. Inhalable formulations that can deliver intact CAMP to conductive airways while

protecting it from interactions with mucus and bacterial biofilm are urgently needed. This work aimed to create and manufacture nano-embedded microparticles (NEMs) for long-term CAMP delivery in the lungs [79]. Colistin (Col) has been used as a model CAMP in creating NPs made of poly(lactide-co-glycolide) and emulsion/solvent diffusion. PVA and Chitosan were used to modify the surface properties of nanoparticles, which in turn increased their transport through artificial cystic-fibrosis mucus. NPs were spray-dried in lactose or mannitol to produce NEM, a long-term stable dosage form suited for NP inhalation. A breath-activated dry powder inhaler was used to deliver the most promising NEM formulations based on their flow, bulk, and NP dispersion properties. Compared to free Col, NEMs loaded with Col were shown to be more efficient in eradicating *P. aeruginosa* biofilm and have a longer-lasting impact on biofilm removal. This action was most likely due to NPs' capacity to enter bacterial biofilm and maintain Col release inside it, as confirmed by confocal examination. Overall, our findings suggest that the proper creation of PLGA NPs provides an attractive technical strategy to harness new anti-microbials for *P. aeruginosa* lung infection, such as CAMPs, particularly in CF [79]. On the other extreme, combining silver NPs with aztreonam seemed highly promising. Silver nanoparticles with a diameter of 10 nm were used in conjunction with aztreonam, which resulted in a 50% reduction in thickness and a 98% reduction in biomass [76]. Furthermore, reactive oxygen species (ROS) generation was substantially greater when silver nanoparticles were combined with an antibiotic than when either treatment was administered alone [80].

Even though it is still unclear what is responsible for silver NPs' antimicrobial activity, the consensus is that the majority of damage to bacterial cells in the presence of silver NPs, as with other metal NPs, is caused by reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, which can cause protein and DNA damage in bacteria [81]. In most cases, silver ions created by silver nanoparticles react with thiol groups in proteins, disabling essential respiratory enzymes and resulting in dangerous ROS levels that harm or kill cells [82, 83]. It has also been found that the reactive oxygen species (ROS) formed on the surface of silver NPs may interact with cell membrane lipids, disrupting cell membrane functions [81]. As previously reported [83, 84], silver nanoparticles have also been demonstrated to connect to and enter bacteria's cell walls, increasing the cell wall's permeability and the likelihood of cell death. Because of this, the combination of silver nanoparticles with conventional antibiotics seems to be promising for the treatment of biofilm infections.

CONCLUSION:

In multispecies biofilms, interactions between diverse species are apparent. Research on biofilms should integrate several methodologies to understand these complex ecosystems better. We can learn a lot about biofilm infections by studying the behaviour of different bacterial cells in the multispecies biofilms. Reproducibility is now a problem in biofilm research. Biofilm tests are carried out in various ways by researchers from many fields and organizations, resulting in a wide range of biofilm investigations. This knowledge may lead to the creation of intelligent biofilm engineering, which can design and regulate the growth of biofilms.

A biofilm infection, which may be exceedingly difficult to remove, is the most common cause of long-term infection. In addition, the medical sector has a critical issue with antibiotic resistance, which is exacerbated further by biofilm bacterial infections. Silver nanoparticles' antibacterial properties and potential therapeutic uses have lately increased interest. The antibacterial activities of AgNPs remain mostly unknown despite multiple investigations undertaken over the last year. Several bacteria have been shown to suppress the production of in vitro biofilms at appropriate nanoparticle concentrations. Using AgNPs to treat illnesses caused by biofilm-forming bacteria provides an exciting prospect. Using nanotechnology, antibiotic medication delivery may increase the effect of standard antibiotics on biofilm infections by delivering large dosages of medicines to the biofilm bacteria in a targeted or localized manner. Silver NPs might be an alternative to antibiotics in the treatment of infections. We may expect Silver nanoparticles to play a critical role against biofilm infections in the medical business, whether via prevention, disruption, or eradication.

ACKNOWLEDGMENT

With gratitude, the authors express their appreciation to the Department of Pharmacognosy and PhytoPharmacy, JSS College of Pharmacy at the JSS Academy of Higher Education and Research in Ooty (Nilgiris, Tamil Nadu, India), which provided them with the necessary resources to complete the work.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest

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