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Bacterial Biofilms: A Current Clinical Dilemma and a Promising Therapeutic Target

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ABSTRACT

Bacterial cells in the natural environment and in infections are rarely found in a planktonic state. They are instead arranged in well-organized communities embedded in self-produced extracellular polymeric substances (EPS) called biofilms. The biofilm lifestyle confers a wide range of properties to the residing cells that allow efficient social interaction, permit nutrient availability, and ensure optimum usage of available enzymes and resources. The biofilm structure also permits a high level of tolerance to antimicrobials and host defense mechanisms. This creates a clinical milestone in treatment of biofilm-related infections. The medical consequences of biofilm formation and associated device-related infections (DRI) have been amplified with the widespread use of implanted medical devices. However, the biofilm structure itself represents a promising target in the development of novel antibacterial drugs. Therefore, this review represents an overview on the biofilm properties and the role of the EPS in the biofilm ecosystem. In addition, it emphasises the involvement of the biofilm structure as a therapeutic target in the development of novel antimicrobials and treatment of biofilm-related infections.

Keywords: *Biofilm, EPS, Antimicrobial resistance, Quorum sensing, Persister cells.*

INTRODUCTION

Biofilms are frequently defined as surface adherent bacterial communities enclosed in self-produced EPS refereed as the biofilm matrix [1, 2]. The concept of bacterial biofilms has been expanded to include non-surface attached bacterial aggregates where the bacterial cells may adhere to each other and/or interfaces. For example, adherent populations within the pores of porous supportive media, bacterial flocs in wastewater treatment plants, and mucus-embedded bacterial aggregates from cystic fibrosis patients [1, 3].

Bacterial cells in biofilm ecosystems are not as simple as a sessile form of their free-floating counterparts that adhere to surfaces. Conversely, their proteomic and transcriptomic profiles are extensively different [4]. In fact, the biofilm mode predominates in most of the environmental, industrial, and medical circumstances [5].

In contrast to planktonic bacteria, each bacterial cell -within the biofilm- lives in a well-organized, metabolically cooperative microbial community, with a simple homeostasis and simple circulatory system. In this structure, resident cells

experience localized environmental gradients, providing habitat diversity, resources are captured and enzymes are retained, providing digestive capabilities and social interactions [5, 6].

The biofilm matrix has a great role in this microbial community. First, the EPS immobilizes the biofilm cells, allowing intense interactions between close cells, cell–cell communication, and the development of synergistic micro-consortia. Second, it provides retention of extracellular enzymes so that an external digestive system can be established. Third, it furnishes additional nutrients and energy sources by sequestering nutrients from the water phase. The EPS itself can serve as a nutrient source regardless of its slow biodegradability. Fourth, it keeps all the components of lysed cells available for recycling. For example, keeping DNA as a reservoir of genes for horizontal gene transfer. Fifth, the hardly biodegradable complex structure of the biofilm matrix protects the enclosed organisms against biocides, antimicrobials, pH changes, ultraviolet radiation, and host immune defenses [6-8].

The biofilm matrix represents the largest and defining component of biofilms. The biofilm matrix composition varies greatly within different polymicrobial and single-species biofilms. The most common components include polysaccharides, extracellular DNA, lipids, proteins and extracellular bacterial structures such as flagella, pili and fimbriae [9, 10].

Biofilm formation process and the related medical consequences

The establishment of a mature biofilm on a surface passes through four stages; initial attachment, formation of microcolonies, microcolonies maturation, and finally dispersion [11, 12]. The entire process starts with reversible attachment of a few free-swimming planktonic cells to the underlying living or non-living surface. Later, this attachment becomes irreversible because of the hydrophobic and hydrophilic interactions between the surface and adsorbed cells. Host matrix proteins (collagen, fibronectin, and fibrinogen) can facilitate this adhesion by forming a conditioning film on the surface [6]. The second step involves growth and multiplication of the attached cells into a complex multi-cellular form called microcolonies, which undergoes further maturation to a well-structured mature form equipped with water channels that act as pipelines for nutrient flow through the established biofilm [3, 5]. These phenotypic and architectural changes are associated with multiple gene expression changes, providing several biofilm specific benefits. Interestingly, different gene expression patterns are experienced within the biofilm due to different physicochemical conditions like water and nutrient availability, cell density, pH, and metabolic side products. To ensure spread and stability of the formed biofilm, some bacterial cells are dispersed by either physical detachment or signaling events, returning to the planktonic state. This enables occupancy of new niches [8, 13]. The dispersion step allows the biofilm to escape triggers resulting from the increased size of the microcolonies, including separation of deep layers from the liquid interface and essential nutrients, in addition to accumulation of waste products and toxins. Many physical and chemical factors can trigger the detachment of cells from the sessile biofilm. These include shear stress, degrading enzymes, signaling factors, oxygen and energy source availability [3, 8].

Great financial expenses have been experienced in the medical industry as a consequence of biofilm formation and biofouling in medical devices including contact lenses, urinary catheters, cerebrospinal fluid shunts, prosthetic, dental, and breast implants [14]. Once the device has been inserted, host-derived adhesins aid the start of biofouling by forming a conditional layer that attracts the planktonic bacterial cells to attach onto the implant surface. Medical biofouling gives rise to DRI, device malfunction, implant rejection and associated costly surgical removal and replacement procedures [15]. A wide range of organisms are implicated in DRI, including *S. aureus, S. epidermidis, E. coli, P. aeruginosa, C. albicans*, and *K. pneumoniae* [16-18]. Non-device-related biofilm infections are also widely distributed, representing another health care and financial consequence of undesirable biofilm formation. Non-device-related biofilm infections include periodontitis (infection of the gums caused by *Pseudomonas aerobicus* and *Fusobacterium nucleatum*), rhinosinusitis, and cystic fibrosis [19]. Rhinosinusitis is triggered by bacterial or fungal colonization of the paranasal sinuses that causes acute or chronic inflammation. *P. aeruginosa, S pneumoniae, S. aureus, H influenza*, and *Aspergillus fumigatus* are all implicated in rhinosinusitis [20]. Cystic fibrosis is an inherited condition caused by a mutation in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This leads to accumulation of sticky secretions in the lungs, gut, and pancreas. Symptoms include difficulty breathing and constant coughing up mucous, and the lungs become more susceptible to biofilm infections. In CF patients, lung biofilm infections with *P. aeruginosa* can lead to failure of antibacterial therapy and overall poor prognosis [16, 20].

Properties of biofilm

Bacterial biofilms exhibit many properties that are completely different from the free-living lifestyle. Structural and functional features of the biofilm matrix are the main effectors that control the properties of

different biofilms. These properties include localized nutrient and chemical gradients, trapping and capturing resources, enzyme retention and digestive abilities, and enhanced tolerance to antimicrobials.

Localized gradients and habitat diversity

The biofilm matrix provides stable gradients within the biofilm, creating different subpopulation habitats [21]. This feature is observed in both thick, multilayer biofilms and in relatively small growing biofilms where only a small number of cells have been attached to a surface. The oxygen gradient-for example-affects the arrangement of cells in response to difference in oxygen availability within the biofilm layers. In aquatic habitats, actively respiring aerobic microcolonies are experienced at the surface of the biofilm, where oxygen is more readily available. Deep within the biofilm, oxygen becomes depleted and anaerobic zones are formed [22, 23].

The nutrient gradient is another determinant that controls stratification in aerobic oligotrophic biofilms [24]. Here, the upper layer organisms consume nutrients, resulting in starvation of the lower layer organisms, leading to adaptation to slow growth states like dormant, viable-but-nonculturable cells (VBNC), persister cells or even dead cells. The nutrient gradient is reversed if the nutrients are acquired mainly from the base layer of the biofilm. Other considerable gradients are present in biofilms, including pH and signalling molecules gradients [3]. The pH gradient arises as a consequence of the heterotrophic metabolism of resident cells. While the concentration of Quorum Sensing (QS) molecules is controlled by the distribution of the producing cells within the population [25].

Social communication in biofilms

Social communication behaviors were recorded in biofilm communities, including both cooperative and competitive interactions [24]. Cooperation can be mediated by chemical signals like acyl homoserine lactone (AHL) and other QS signals [26]. Electrical cooperative communication via nanowires (electrically conductive appendages produced by several bacteria) was also recorded [27, 28]. Cooperative metabolism is another example of cooperative interactions within biofilms. For example, the cooperation observed when ammonia oxidizing bacteria become close to nitrite oxidizing bacteria as the former produce nitrite that is further oxidized by the later one [29]. Competitive interactions involve killing mechanisms using antibiotics, bacteriocins, or extracellular membrane vesicles, or impairing the growth of competing organisms by depleting nutrients or QS inhibition [30, 31].

Resource capture by biofilms

The sponge-like nature of the EPS biofilm matrix enables the biofilm to capture resources and nutrients from the underlaying substratum and other surroundings. A wide range of substances can be captured from the surrounding environment and accumulated in the biofilm matrix for further consumption by biofilm cells. Different resources may be absorbed in the aqueous phase of the biofilm matrix or adsorbed to matrix biopolymers. Absorption and adsorption processes are collectively referred as sorption [32]. Different sorption mechanisms and binding sites are used within the biofilms. These binding sites include both cationic and anionic exchangers, which are not compound specific. This allows a wide range of nutrients and toxic substances to be accumulated in biofilms. If the sorbed substances are not degraded, they will be released into the surrounding media whenever the concentration gradient allows. Otherwise, they will be reserved for later decomposition by the biofilm. Biofilms have the ability to trap and incorporate suspended organic and inorganic solid particles as well. Inorganic particles include clay and silicates [7, 25, 33, 34].

Binding sites for metal ions like calcium, iron, and manganese allow their sorption and accumulation in the matrix. The heavy metal sorption capacity of biofilms finds some applications in biotechnology, including the decontamination of wastewater [35]. However, in activated sludge, it creates a problem when the sludge is used as a fertilizer due to the accumulation of metal ions like lead, cadmium, and cupper [36].

Capturing and recycling of dead cell debris is another way to ensure nutrient availability for the biofilm's consumption, as dead cell debris remains in the matrix and acts as a nutrient for healthy cells [37].

Enzyme retention

Compared to planktonic bacterial cells, biofilms utilize their extracellular degradative enzymes much more effectively. The extracellular enzymes secreted by the biofilm cells are retained within the biofilm matrix, creating an external digestive system [38]. These matrix-bound enzymes represent a reserve that is accessible to all cells in the population, even in a mixed-species consortium. In natural biofilms, the extracellular enzymes interact with the matrix components, leading to stabilization of the enzymes and persistence of their enzymatic activity [38, 39]. On the other hand, the extracellular enzymes secreted by free-living bacterial cells diffuse away and become diluted in the aqueous surroundings.

The matrix-retained extracellular enzymes have various roles, including matrix modulation and continuous restructuring, biofilm detachment and dispersal. Furthermore, biofilm associated enzymes may act as virulence factors [21, 38].

Enhanced tolerance to antimicrobials

Bacterial biofilms are much more tolerant to antimicrobials compared to planktonic cells. It was formerly considered that the biofilm matrix acts as a diffusion barrier that prevents penetration of antimicrobials, resulting in reduced susceptibility. This was supported by the innate ability of the EPS in several biofilms to prevent antibiotic penetration. However, this phenomenon is not universal as some antimicrobials have been shown to diffuse freely within established biofilms and the EPS does not hinder their diffusion [40]. For example, ciprofloxacin and ampicillin can successfully penetrate *K. pneumoniae* biofilms and reach deep biofilm cell layers. Another example is the free diffusion of ciprofloxacin through *P. aeruginosa* biofilms. The same behavior was reported in the diffusion of tetracycline within *E. coli* biofilms. However, many of these antibiotics are still ineffective in eradicating the biofilms, indicating other mechanisms contributing to the enhanced biofilm tolerance to antimicrobials [25, 41].

Tolerance to antimicrobials in biofilms can be attributed to the entrapment or inactivation exhibited by the matrix, the slow growth of the biofilm cells, expression of efflux pumps, and the efficient horizontal gene transfer [42]. The EPS matrix can inactivate antimicrobials by chelation, inactivation, or enzymatic degradation and thus decrease their effective levels to sublethal concentrations [43]. This may promote selection for antimicrobial resistance within the biofilm cells.

Beyond conventional antibiotics, toxic metals can also be inactivated by the biofilm matrix. For example, *Erwinia amylovora* biofilms can escape toxic copper stress through complexation with polysaccharides in the matrix [44]. Toxic metals can also be inactivated by extracellular signalling, reaction with siderophores, metal immobilization and complexing, and genetic mutations [25].

The reduced growth rate plays an obvious role in the enhanced biofilm antimicrobial tolerance. As mentioned previously, the oxygen and nutrient gradients within the biofilm habitat create zones of slowly growing bacterial cells represented by considerable numbers of stationary cells, VBNC cells, and persister cells (extremely tolerant, dormant, microbial subpopulation). These dormant cells have a decreased susceptibility to antimicrobials that depend on the metabolic activity of bacterial cells for their actions [45-47].

Persisters are simply a phenotypic variant that is tolerant to antibiotics but not a resistant mutant. Persisters are present in biofilms in small numbers. However, they afford protection against the immune system to the whole community and support the persistence of infection. Relapsing of biofilm-related infections is directly related to the presence of persister cell subpopulations as they can remain after antibiotic treatment regardless of the concentration utilized, and once treatment ceases, they start to repopulate and establish a new biofilm [47, 48].

Horizontal gene transfer is much more efficient in biofilms compared to free-living bacterial cells due to the high cell density, increased genetic competence, and accumulation of mobile genetic elements provided in the biofilm lifestyle [49]. Additionally, the biofilm matrix ensures efficient cell-to-cell contact by offering a stable physical environment. The simplicity of the uptake of resistance genes within the biofilm cells is considered as one of the mechanisms by which the resistance of the biofilm to antimicrobials can be boosted [25, 50].

Bacterial biofilms as a therapeutic target

The majority of human bacterial infections (65–80%) are correlated to biofilm formation [11]. Treatment of biofilm related infections, including localized chronic infections, is considered a massive challenge in the clinical settings. Many factors can lead to treatment failure, including biofilm characteristics, including the complex physical and biological properties of the biofilm, antibiotic tolerance, metabolic dormancy, and various microbial genetic and molecular factors. In addition to the expanded use of implanted medical devices [17]. Finally, the polymicrobial nature of most chronic biofilm-associated infections, including cystic fibrosis lung and diabetic foot infections [51-53]. Infectious biofilms frequently involve multi-species interactions with a diverse range of bacteria or even fungi that coincide in a mixed biofilm infection [54]. Moreover, it's common for biofilm infections to relapse following long periods of clinical dormancy. The expanding knowledge about the nature and composition of microbial biofilms has proposed new strategies to treat these challenging infections [55]. Treating such infections must involve a combination of treatments that target various elements of the complex biofilm microenvironment.

As mentioned earlier, the establishment of a mature biofilm passes through four distinct stages: the attachment and adhesion, followed by early biofilm formation, then the biofilm maturation, and finally the dispersion stage. All these stages can be targeted for managing infectious biofilms. In one policy, the initial attachment phase can be interrupted by targeting the cell-surface interactions or the associated adhesins (appendages, proteins, and EPS). Targeting EPS production and cellular division would interfere with the early stages of biofilm formation. Interfering with established biofilms may include physical removal, EPS matrix degradation, targeting the establishment of microenvironments and social interactions, and eradication of dormant cells. Lastly, the biofilm

dispersion can be stimulated by remodelling the EPS matrix or triggering the dispersion mechanisms [55].

Preventive approaches

Following the guidelines and standard procedures for device implantation and handling and the immediate removal of unnecessary medical devices would help to prevent microbial contamination, adherence, and subsequent biofilm formation and development of DRI. Another preventive measure is to use a prophylactic systemic antibiotic during device insertion. This is recommended in the case of surgically implanted devices, including orthopedic and cardiac devices. Antibiotics are injected before skin incision to eliminate any microorganisms that resist skin disinfection [56, 57].

Another evident preventative tool is to alter the surface of implanted medical devices to prevent bacterial attachment. Surface modification is either directly using antibacterial surfaces or with the aid of antibacterial coating. This approach has shown significant potential for preventing DRI [58]. In this context, a wide range of coating materials can be applied, including nanoparticles [59-62], antibiotics [63, 64], and cationic polymers [63, 65, 66].

EPS-targeting strategies

Many factors influence the definite structural composition of the biofilm matrix, including the microorganism(s) in residence, local shear forces, substrate accessibility, and the host environment [67]. As mentioned previously, the EPS matrix functions to promote surface adherence, cell–cell adhesion, and aggregation. In addition, it permits mechanical consistency and affords protection against host defence and antimicrobial treatments. The EPS matrix dynamically modulates the chemical and nutrient gradients [10, 25, 55]. Therefore, targeting the EPS matrix would represent an effective approach to disperse bacteria and eliminate biofilms [68].

Matrix degrading enzymes

Targeting the EPS structure using matrix-degrading enzymes can weaken the biofilm cohesion and disperse the biofilm bacteria that would improve the efficacy of antimicrobial agents and enhance host defence mechanisms. For example, the *in vitro* use of glucanohydrolases, glycoside hydrolases, and DNases against established biofilms enhanced the antimicrobial delivery and killing by antibiotics and antimicrobial peptides [55].

In this context, both dextranase [69] and mutanase [69, 70], were proved to have effective EPS degrading activity against *S. mutans* plaque biofilms. In addition, in combination, they can act synergistically and selectively against the cariogenic bacteria [70]. Dispersin B [71] and other glycoside hydrolases including PelA, PslG, and Sph3 are able to disrupt formed *S. aureus* and *P. aeruginosa* biofilms *in vitro* [71-74]. Other examples include α-amylase and cellulase that succeeded in degrading a mixed-species *S. aureus* and *P. aeruginosa* biofilm in mouse models of chronic wounds [75]. However, this approach is challenged *in vivo* by poor retention and stability.

DNase I has also revealed efficacy in early biofilm disruption *in vitro* and *in vivo* [76]. The therapeutic use of dornase alfa (recombinant human DNase I) in cystic fibrosis patients and early lung disease revealed decreased sputum viscosity, improved lung function, and a decreased risk of exacerbation, with a significant reduction in the rate of lung function decline in children [77, 78]. In a recent study, a cationic liposome loaded with a mixture of DNase I and proteinase K exhibited potent antibiofilm activity and eliminated skin and catheter infections caused by *Cutibacterium acnes* [79]. The antibiofilm activity of DNase I was further enhanced through a nano-formulation using silver-doped mesoporous silica nanoparticles [80].

EPS-targeted antibodies

Another promising approach to control biofilm-related infections is to use antibodies that target specific EPS components. Psl is an antibody-accessible, serotype-independent antigen which is extensively distributed among *P. aeruginosa* clinical isolates. Psl-specific antibodies enhanced opsonophagocytic killing of *P. aeruginosa*, inhibited the *in vitro* adherence to lung epithelial cells, and displayed prophylactic protection in different *P. aeruginosa* infection animal models [81-83].

Antibodies targeting the DNA-binding proteins (DNABII family) have proved efficacy in controlling bacterial biofilms [84]. DNABII has a crucial role in conserving the structural constancy of eDNA. For example, antibodies against *E. coli* IHF (integration host factor) are cross-reactive and can bind to DNABII in different bacterial species, leading to destruction of formed biofilms. In combination with antibiotic therapy, targeting DNABII is effective *in vivo* against oral biofilms, uropathogenic *E. coli*, *P. aeruginosa*, and MRSA biofilms [85-87]. Targeting DNABII with monoclonal antibodies also effective against otitis media caused by *Haemophilus influenzae* [88].

Metabolic interference

Interference with the natural bacterial metabolism has been shown to influence both biofilm metabolism and development. This can be achieved using different molecules, including-for example-exogenous amino acids and gallium.

The amino acid l-Arginine (l-Arg) altered the polymicrobial (*S. mutans*, *S. gordonii*. and *Actinomyces naeslundii)* biofilms and suppressed the growth of the cariogenic *S. mutans.* This was attributed to its pH modulatory effects (through deamination by the arginolytic *S. gordonii*) and its ability to repress genes that are responsible for EPS and bacteriocin production in *S. mutans* while inducing hydrogen peroxide production by *S. gordonii* (used against *S. mutans*). In the same way, l-Arg can disrupt other multispecies oral biofilms [89-91]. Another example is the amino acid l-methionine (l-Met) that can upregulate different DNase genes and hence enhance degradation of eDNA in the EPS matrix of *P. aeruginosa* biofilms, triggering its disassembly [92]. l-Met activity in cystic fibrosis patients could be enhanced through cotreatment with the cystic fibrosis transmembrane conductance regulator potentiator (ivacaftor) [93].

Another promising approach involves interfering with the iron metabolism, which is crucial for biofilm formation in several pathogens, including *P. aeruginosa and S. aureus*. Gallium which is chemically similar to iron, can interfere with all the iron-dependent pathways, including biofilm formation [94-96].

Targeting the c-di-GMP pathway

The intracellular secondary messenger nucleotide c-di-GMP has a key role in biofilm development and regulation in both Gram-positive and Gram-negative bacteria [97]. Levels of c-di-GMP is governed by diguanylate cyclase and phosphodiesterases. Therefore, it represents an attractive target to control biofilms formed by different species. The use of phosphodiesterase was proven to reduce c-di-GMP levels and disperse established biofilms *in vivo* [98-100]. This strategy has many limitations, including the complexity of c-di-GMP regulation that obscure its control, its difficult to attribute specific effects on biofilms *in vivo* because of the multiple roles of c-di-GMP including its role as stimulator of host immunity.

Nitric oxide (NO) can also be used to modulate c-di-GMP levels and mediate biofilm dispersion in *P. aeruginosa* and other organisms [101]. In a primary clinical study, gaseous NO was used in a picomolar to nanomolar concentration range in a small number of cystic fibrosis patients. It was shown to decrease the *P. aeruginosa* biofilm aggregate in their sputum without adverse effects [102].

Targeting dormant cells

Targeting cellular pathways to disperse biofilms requires metabolically active cells. Therefore, eradication of dormant and metabolically inactive cells requires different intervention that can disrupt cells instead of interfering with cellular processes. Physical disturbance of biofilms has been experienced using irrigants such as hypochlorite and hydrogen peroxide in wounds and periprosthetic joint infections. This approach was limited by cytotoxicity concerns that govern longer exposure and often lead to therapeutic failure [103, 104].

In the same context, dormant persister cells in Gram-positive bacteria were targeted using the acyl-depsipeptide antibiotic (ADEP4). ADEP4 can activate the bacterial ClpP protease that breaks down essential proteins. In this way, the bacterial cells digest themselves and degrade their own biofilms. However, ClpP is not an essential enzyme, and ClpP-defecient mutants are not sensitive to ADEP4 treatment [105].

Many other compounds have been found to possess anti-persister activity, including synthetic peptides like SAAP-148 and natural analogs like Pexiganan [106, 107].

Targeting quorum sensing

Another promising target to control infectious biofilms is the QS systems that play fundamental roles in the regulation of diverse bacterial biofilms. Sensing the bacterial quorum requires a signalling molecule that binds to its corresponding transcriptional regulator and hence regulates transcription of the downstream genes. QS naturally regulates numerous virulence determinants in pathogenic bacteria. Consequently, various quorum sensing inhibitors (QSIs) have been assessed for their antibiofilm activity *in vitro* and *in vivo*. Different QSI strategies have been evaluated in both bacterial and fungal biofilms, including targeting the Gram-negative AHL-based QS or the auto-inducing peptide (AIP)-based QS systems in Gram-positive bacteria [108-111].

In recent studies, different natural compounds showed anti-QS and antibiofilm activity. For example, Hamamelitannin, which is a natural product present in the leaves and the bark of *Hamamelis virginiana* (witch hazel). Hamamelitannin inhibits *Staphylococcal* biofilm formation and virulence [112-114]. Thymol-carvacrol-chemotype (II) oil and other essential oils of aromatic plants exhibited antibiofilm and anti-QS activity against *E. coli* and *S. epidermidis* [115]. In another study, fungal extract (*Blastobotrys parvus* PPR3) showed antibiofilm and anti-QS activity against *P. aeruginosa* [116]. Other research groups developed synthetic anti-QS compounds that have antibiofilm activities [117-119]. Several metal nanoparticle preparations also showed anti-QS and antibiofilm activities [120-123].

Clinical interference with the cell signalling mechanisms in controlling infectious biofilms encounters many challenges due to the complexity of cell signalling networks. Additionally, the QS molecules can be sequestered within the EPS matrix, hindering the access of the QSIs to the site of active QS signalling and limiting the effect to highly localized

areas within the biofilm structure. However, such an approach can be used in combination with other strategies [55, 124].

Antimicrobial peptides (AMPs)

AMPs are naturally produced by almost all living organisms, including bacteria (bacteriocins), fungi, plants, and animals as a component of their innate immune systems. AMP's main role is to kill invading pathogens, including bacteria, fungi, viruses, and even parasites. Most AMPs have broad-spectrum activities as they target several bacterial enzymes, pathways, or structures [125]. Unless not fully understood, their mechanism of action is often linked to permeabilization of the cytoplasmic membrane and inhibition of protein folding or enzyme activity [47, 126]. AMPs display potent antimicrobial activity against multidrug resistant bacteria and slow-growing or dormant biofilm-forming cells and are less likely to induce resistance compared to existing antibiotics [126].

LL-37 is a human AMP synthesized and secreted by the epithelial cells and some immune cells, including natural killer cells, macrophages, dendritic cells, and neutrophils. LL-37 is one of the first AMPs that was shown to have antibiofilm activity in different stages of biofilm formation, starting from the prevention of bacterial cell attachment, inhibition of biofilm formation, and disruption of mature biofilms [127]. LL-37 antibiofilm activity extents to include different aerobic and anaerobic bacteria like *S. aureus, S. epidermidis, P. aeruginosa , S mutans, Streptococcus sanguinis, Actinomyces naeslundii, Veillonella parvula, Parvimonas micra* and *Fusobacterium nucleatum* [47, 127, 128].

Many AMPs have synergistic effects when used in combination with antimicrobial treatment. For example, G10KHc showed synergistic activity when combined with tobramycin for treatment of *P. aeruginosa* biofilms and BMAP-28 combined with vancomycin for combating biofilms formed by *Enterococcus faecalis* and *S. aureus* [126]. The broad-spectrum activity of AMPs can be altered synthetically to achieve species-specificity using dual functionally independent moieties (a broad-spectrum killing moiety and a species-specific binding moiety). This approach is used to remove specific pathogens and promote a healthy microbiome. This policy can be applied for removal of the pathogenic *S. mutans* from oral multispecies biofilm communities. The use of AMPs in combating biofilm infections has many drawbacks including the possibility of binding to the EPS matrix molecules, which diminishes their efficacy. In addition to their liability to microbial protease digestion and elevated manufacturing cost [55, 129]. However, the therapeutic efficacy and bioavailability of AMP have been significantly improved through immobilization, nano-formulations, and different drug delivery formulations [127, 130, 131].

Several AMPs have proved efficacy in the treatment of polymicrobial infections. For example, DRGN-1 [132], and Pexiganan-nisin [133] for treatment of *S. aureus* and *P. aeruginosa* mixed infections, and Tet213 for treatment of mixed wound infections with *E. coli and S. aureus* [134]. Furthermore, some AMP showed efficacy against mixed bacterial-fungal [135] or bacterial-viral [136] coinfections.

Fatty acids

There is a wide variety of saturated and unsaturated FAs distributed in nature. They are synthesized and utilized by a wide range of living organisms, including bacteria, yeasts, algae, insects, fish, and plants. In addition to being fundamental for the cell membrane structure, they act as substrates for triacylglycerides, esters, phospholipids, and cholesterol biosynthesis. Producing organisms also use FAs to control the microbial community and protect themselves against pathogens [137, 138]. For example, the human skin commensals *Cutibacterium acnes* and *S. epidermidis* hydrolyze sebum triacylglycerides, releasing FAs. The released FAs have broad spectrum antibacterial activity that suppresses colonization by invading bacteria [139].

Different FAs can selectively inhibit or disrupt biofilms formed by several microbial pathogens, including *P. aeruginosa*, *S. aureus*, *Serratia marcescens*, *Burkholderia cenocepacia*, *Vibrio* spp., and *C. albicans* [138]. Despite that their mechanism of action is not fully understood, FAs affect different microbial pathways including QS-regulated genes (e.g., synthesis of toxins, fimbriae, hyphae, etc.) and non-QS targets (efflux pumps, oxidative stress, and ergosterol synthesis). At high concentrations (above MIC), FAs act on multiple cellular targets, resulting in broad-spectrum antimicrobial activity. While, at lower concentration ranges they act as antibiofilm agents [138, 140, 141].

Cis-2-unsaturated FAs can serve as diffusible signal factors (DSFs). DSFs are QS signaling molecules used by Gram-negative bacteria. The first member of the DSF family is the unsaturated FA cis-11-methyl-2-dodecenoic that was first reported in *Xanthomonas campestris* and was found to induce dispersion of its own biofilm [142]. Later, other Gram-negative bacteria, including *P. aeruginosa*, *Stenotrophomonas maltophilia, Xylella fastidiosa*, and *Burkholderia* spp., were also found to secrete cis-2-unsaturated FAs to regulate biofilm formation and other virulence determinants [138, 143]. These DSFs include cis-11-methyl-2-dodecenoic acid, cis-2-decenoic acid cis-2-dodecenoic acid, cis-9-methyl-2-decenoic acid cis-10-methyl-2-dodecenoic acid. Besides DSFs, DSF-like

molecules such as trans-2-decenoic acid, cis-9-octadecenoic acid, and 10-Methyl-dodecanoic acid were found to prevent the primary adhesion and biofilm formation by several bacterial and fungal species [138, 144].

The clinical use of FAs in disrupting microbial biofilms deals with many challenges, including their intrinsic solubility, hydrophobicity, odor, and toxicity. This can be addressed pharmaceutically using technologies like conjugated FAs, drug delivery devices, polymeric nano-capsules, nano-carrier systems, and liposomes [138, 145, 146]. Another limitation is related to the importance of selecting the proper FA concentration to use in a specific environmental condition. The antibiofilm and antibacterial natures of FAs are largely dependent on the concentration and the surrounding environment. Furthermore, some pathogens can significantly escape the FAs antibiofilm effects through different pathways, including modifying FA structures using lipases, desaturase, esterase, and other FA-modifying enzymes. FAs antibiofilm effects can also be precluded through using multiple pathways, including efflux pumps, membrane stabilization, and capsule formation [138].

CONCLUSION

Coinciding with the universal spread of MDR, the application of the medical devices had expanded and became essential and irreplaceable in several medical interventions. This creates an urgent clinical dilemma regarding the treatment of the DRI. The complex and variable nature of the biofilm matrix confers several properties to the residing bacteria and adds more complications to this crisis. This review represents a brief illustration of the bacterial biofilms as virulence factors and at the same time promising therapeutic targets.

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Contributions

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