Biofilm Prevention and Therapy: Alternatives to Standard Antibiotics

Subjects: Infectious Diseases

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In order to combat the global health crisis of escalating antibacterial resistance, guidelines on responsible antimicrobial stewardship are urgently required. Yet, currently there is no international consensus. Tackling discrepancies that may arise when implementing novel antibiotics is critical to their longevity of use. A wide range of antibiotics previously heavily used is no longer effective due to elevated minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) doses for treatment. Thus, there is a pressing need to develop effective way to prevent biofilm from forming as well as to carefully apply alternative therapies to standard antibiotic treatments.

Biofilm | Antibiotic | Antibacterial resistance | Antimicrobial stewardship | Alternative therapy | Anti-biofilm agent | Staphylococcus | MRSA

1. Introduction

A biofilm is a complex surface-adherent aggregate of bacteria bound together by a self-produced polysaccharide extracellular matrix (ECM). This impervious barrier protects the underlying bacterial community from attack by antimicrobials, shear forces and the immune system. In the last few years, in response to the increasing public health threat posed by antibiotic resistance, considerable advances have been made in developing anti-biofilm prevention and treatment measures that can be applied at the bedside [1]. Further fundamental research is needed to identify and validate novel approaches against the key targets of antimicrobial resistance, notably to methicillin and vancomycin [2].

Most biofilm prevention strategies are predicated narrowly on vaccines that target surface antigens or on surface coating of bacteria with chemical compounds or antibiotics. Meanwhile, therapy targets are broader, spanning all steps of biofilm formation from adhesion to dispersal. Notable approaches are the use of nanoparticles, laser therapy, probiotics, bacteriophages, and antibodies, each of which has strengths and weaknesses. As their efficacies and specificities are different, care should be taken in considering the treatment most appropriate for a patient among those available [3].

2. Prevention

2.1 Vaccines

Ongoing research aims to identify a suitable vaccine candidate to prevent *Staphyloccus aureus* biofilm-related infections, which has served to highlight the emergence of antibiotic-resistant strains. Although preliminary results have shown promise, a potential candidate has yet to reach advanced stages of development. Examples can be seen in experimental vaccines against *S. aureus* iron surface determinant B (IsdB), polysaccharide-intercellular adhesins (PIA), FnBP and ClfB, all of which fail to target biofilm [4][5][6]. Unfortunately, most of these constructs that target capsular polysaccharides have stalled in the phase II clinical trial as they do not elicit sufficient protective immunity. Nonetheless, their capacity to ameliorate biofilm conditions can be improved by pairing with Freund's adjuvant [7]. Similarly, conjugating PIA with diphtheria toxoid produces a strong adjuvant effect. Pre-clinical *in vivo* trials on PIA-based constructs showed promise [4]. Not all clinical isolates, however, express these virulence factors. Evidently, anti-biofilm immunization shows early potential, but requires further research. It is critical to consider precautions when designing passive *S. aureus* vaccines. Of note are the presence of multiple *S. aureus* virulence factors, knowledge gaps surrounding immunity against *S. aureus* and the need for information from human trials [8][9][10].

2.2. Antibody-Based Approaches

Harnessing biofilm-penetrating antibiotics is another promising way to prevent biofilm formation. These act at several different levels including attachment and targeting mature biofilm. Many attempts to treat bacterial infections using antibodies have targeted biofilm. TRL1068 was designed against DNABII epitope, an eDNA, with promising results [11]. Likewise, polyclonal antibodies tested against PhnD antigen showed an ability to inhibit biofilm development by both *S. aureus* and *S. epidermidis* [12][13]. Monoclonal antibodies to FnBP and ClfA, when combined with antibodies against the membrane-disrupting alpha-toxin, prevented biofilm formation. The antibody targets FnBPA, SasG, Atl and Atl-Amd have been tested only *in vitro*, while ClfA, Can and Atl-Gmd have undergone *in vivo* trials with satisfactory results [12].

3. Therapy

3.1. Biofilm-Degrading Enzymes

Dispersin B is an enzyme that is produced by *Aggregatibacter actinomycetemcomitans.* It degrades mature biofilm and thus may provide a novel therapy [14]. Similarly, rhDNase has a potent effect on eDNA and so could be exploited to either prevent or treat infection. Additionally, it increases the sensitivity of biofilm to antibiotics such as tobramycin. Dispersin B shows similar biocidal properties towards biofilm when paired with tigecycline or vancomycin [15]. Moreover, dispersin B can act alongside proteases to improve treatment outcomes [16].

3.2. Probiotics

Microorganisms that live beneficially within the human host's body are described as "probiotic", a term particularly ascribed to commensal gut microflora. They can interfere with potentially pathogenic bacterial growth through disrupting the biofilm community by competitively inhibiting attachment to shared substrates. Probiotics are a

preferred choice to eradicate biofilm-forming opportunistic bacterial infections as they have a varied arsenal of antimicrobial molecules including organic acids, enzymes, surfactants and bacteriocins. Interference with biofilm occurs at different levels including attachment, quorum sensing, pathogen maintenance and disturbance of structural integrity. Another feature of probiotic species is that they compete effectively with other bacteria for the same ecological niche, and thereby prevent colonization by potential pathogens [17][18][19].

Several strains of the popular probiotic dietary supplement *Lactobacillus acidophilus* show anti-biofilm activity, and therefore are effective agents against *S. aureus*, including that produced by methicillin-resistant *S. aureus* (MRSA). Additionally, attachment, growth and formation of *S. aureus* biofilm is disturbed by *Lactobacillus plantarum*, *Limosilactobacillus fermentum* and *Pediococcus acidilactici*, each of which inhabits the human digestive tract. Among other probiotics with a potent activity towards bacterial biofilm are *Bifidobacterium lactis*, *B. longum*, *Lactobacillus brevis*, *L. casei*, *L. delbrueckii*, *L. fermentum*, *L. pentosus*, *L. rhamnosus*, *L. salivarius*, *L. sporogenes*, *Streptococcus oralis* and *S. salivarius.* Of these, *L. brevis* and *L. plantarum* were effective against *S. aureus* biofilm *in vitro*. Additionally, *in vivo* trials showed a protective effect of using *L. fermentum* to treat biofilm. Probiotics can be exploited for both prevention and treatment, but further research is needed to optimize efficacy [17][18][19][20][21][22][23].

3.3. Rhamnolipids

A number of alternative agents are being explored for their potential to treat biofilm (**Table 1**), primarily those formed by MRSA. Rhamnolipids are naturally occurring glycolipid biosurfactants that are produced predominantly by *Pseudomonas aeruginosa*. They are harmless to humans and may thus be used in prescription medicines. This feature makes them an attractive candidate therapy for biofilm. Efficacy varies depending on differences in environmental conditions and in nutrient source and level [24][25][26]. In one study, rhamnolipid treatment removed 89% of biofilm attached to a skimmed milk-based agar substrate, but only 35% grown on nutrient medium, due to differing proportions of carbohydrate [27]. Rhamnolipids can disrupt biofilm in combination with caprylic acid and sophorolipids [28][29]. Mono-rhamnolipids have a bacteriostatic effect towards biofilm, while di-rhamnolipids show bactericidal properties [30]. Not only can formation of biofilm be prevented at low concentrations of caprylic acid, mature biofilm [31].

Table 1. Properties of different anti-biofilm agents.

Anti-Biofilm Agent	Mechanism of Action	Level of Interruption	Advantages	Disadvantages	References
Rhamnolipids	Disrupt biofilm	Adhesion Maturation process	High surface activity Biodegradability Low toxicity	Limited production Increasing usage is a threat to synthetic surfactants	[27][32]
Photodynamics	Affect bacterial LPS, endotoxin	Mature biofilm	Synergic effect Strong treatment	Thermal damage Antibacterial	[33]

Anti-Biofilm Agent	Mechanism of Action	Level of Interruption	Advantages	Disadvantages	References
	and cell differentiation			resistance Surface modification	
Nanoparticles	Transport drugs	Adhesion and mature biofilm	Small size Higher surface area to volume ratio	Toxicity	[34]
Bacteriophages	Disrupt biofilm	Mature biofilm	Specific for targets Effective against resistant strains	Further studies required Potential threat to human health	[35]
Antimicrobial peptides	Increase permeability of cell membrane	All three phases	Less chance of resistance Strong antibacterial activity	Further *in vivo* verification required Synthesis and purification are challenging	[36]
Antibodies	Help innate immune system	Adhesion and mature biofilm	Produce vaccine Prevention therapy	Further studies required	[12]
Phytochemicals	Reduce cell adhesion and disperse biofilm	Mature biofilm and dispersal	Natural compounds Strong antimicrobial agents	Poor solubility in aqueous media Further *in vivo* verification required	[37][38]
Chelators and Sulfhydryl Compounds	Decrease bacterial interaction and decrease PIA/PNAG	Adhesion	Potent antibiotic activity	Cytotoxic and genotoxic effects	[39]
Laser Therapy	Oxidative stress and disrupt bacterial cell wall	Mature biofilm	Boost antibiotic efficacy	High temperature in host tissue Cellular damage Further studies required	[40][41]
Enzymes	Target ECM and cell wall and increase chemical reaction	Adhesion and mature biofilm	Harmless to humans	Potential for activating immune system Further studies required	[16][42]

3.4. Photodynamic Therapy (PDT)

Established over a century ago, its common use developed only recently in response to heightened antibiotic resistance rates. PDT involves non-toxic photosensitizers whose activity is accelerated in the presence of oxygen, which can cause oxidative stress and cytotoxicity. Furthermore, activation takes place in the absence of oxygen through photoinactivation against anaerobic bacteria. The antibacterial mechanism is to target cell membrane, bacterial DNA, or enzymes [33].

This may be used to treat dental infections via oxidative damage of biofilm. Applying a low-power laser and photosensitizer in tandem is more beneficial to prevention of oral inflammation than to the detoxification of implant surfaces [43][44]. Combination therapy with antiseptics may boost PDT efficacy [45]. Successful attempts were made using photoditazine, fotoenticine and methylene blue to treat biofilm of *Streptococcus mutans*, *P. aeruginosa* and MRSA [46][47]. In another *in vitro* study, synergism between antibiotics, indocyanine green and EDTA-mediated PDT enhanced eradication of biofilm in MRSA-related infection [48]. PDT is considered as an alternative treatment for biofilm, specifically when it is combined with antibiotics or other inhibitors such as an efflux pump inhibitor or quorum sensing inhibitor. However, more *in vitro* and *in vivo* trials are needed [33].

3.5. Nanoparticles and Nanomaterials

These have recently improved as an alternative method for biofilm treatment. Various classes of nanomaterial are used including carbon-based nanomaterials, polymeric nanoparticles, nanoemulsions, nanocomposites, lipid nanoparticles and metallic oxide nanoparticles. Another, “smart nanomaterial”, has the potential to regulate drug release and alter its characteristics. Nanoparticles can deliver drugs to the site of infection. In addition, their simple preparation and flexible chemical formulation makes them a potential delivery tool for biofilm therapy. Nano-attapulgite, nano-TiO_2, nano-Ag and SiO_2, to name but a few, have shown antimicrobial effects when incorporated in food products [34][49][50][51].

Magnetic responsive nanomaterials are commonly used in magnetic resonance imaging. Activated by rising temperature, they can disperse cells embedded within biofilm. Recently, selenium and iron oxide nanoparticles in Galinstan (a gallium-indium-tin alloy that is liquid at room temperature) showed good anti-biofilm activity [52][53]. Nanomaterials that are responsive to light (e.g., DNase–AuNCs), pH (e.g., chitosan) or enzymes (e.g., micelles) exhibit anti-biofilm activity through dispersing encapsulated bacteria, weakening biofilm matrix and reducing biofilm mass, respectively [54][55][56][57][58].

When applying nanomaterials a few factors should be considered. Firstly, translating *in vitro* trials to *in vivo* conditions may be challenging due to interaction with bacteria in the host body. The second point is insufficient knowledge of nanoparticle toxicity. Additionally, producing low-cost products and boosting efficiencies [34]. Regarding cytotoxicity, nanoparticles are responsible for various bioeffects including oxidative stress and autophagy [59]. For nanomaterials, it is the cell type, size and composition that determine the level of cytotoxicity and hence the fate of the cell [60].

3.6. Bacteriophages

Recently, bacteriophages were introduced as another potential approach. They may be described simply as viruses that can infect bacteria. Lytic phages, which kill the target cell through their replication, are well suited to therapeutic applications. Their small size allows permeation of the biofilm matrix. Additionally, they produce degradative enzymes that attack the ECM. In contrast to antibiotics, the efficacies of which are higher against planktonic cells, bacteriophages are more effective against bacteria within biofilm mass [61]. High specificity and low risk of resistance are further advantages of bacteriophage therapy [62].

Applications of phage therapy to biofilm treatment include phage-derived enzymes, modified phages, phage cocktails and combining phages with antibiotics. Careful attention should be paid to the specific characteristics of phages, such as their diffusion, penetration, and propagation [35][63]. Phage-derived lysin and depolymerase enzymes are introduced by lytic phages. LysCSA13, which is an *S. aureus* virulent bacteriophage CSA13 endolysin, under certain circumstances shows high antimicrobial activity against *S. aureus* [35][64]. Other bacteriophage lysins, such as CHAP(K), lysH5, phi11 and lysK, also show impressive anti-*S. aureus* properties [65][66][67]. Promising *in vitro* and *in vivo* results were attained when applying Csl2 against *S. suis* in zebrafish [68], as well as from testing the depolymerase phages Dpo7 and Dpo42 on *Staphylococcus spp.* and *Escherichia coli*, respectively [69][70].

Experimental use of the second type of bacteriophage against biofilm, genetically modified phage, has been highly successful. Examples are the *T7 E. coli* and modified *ΦEf11 Enterococcus faecalis* phages. The former is a phage that acts by expressing hydrolase, which achieved a more than 99% elimination rate [71][72]. Finally, combining phage therapy with antibiotics is a novel approach with higher efficacy compared to applying either treatment on its own. This is attributed to phage-antibiotic synergy, a phenomenon in which phage virulence is enhanced by exposure to a sub-lethal dose of antibiotic [73][74]. Studies using *Sb-1 S. aureus* and *T4* phage showed a synergistic effect on antibiotic efficacy against biofilm [75][76].

3.7. Antimicrobial Peptides (AMPs)

AMPs are natural or synthetic oligopeptides that form part of the innate immune response of different organisms, and which have a wide range of inhibitory effects. Several antimicrobial peptides have been explored as novel treatment strategies. The twin public health challenges of biofilm-related infections and increasing prevalence of antibiotic resistance have led to the application of endogenous AMPs and antibodies that can each play a role in both treatment and prevention. AMPs show antibacterial activities through various mechanisms including interfering with bacterial cell signaling, destroying the cell membrane, and interrupting the bacterial alarmone system [77][78].

One of the first developed anti-biofilm peptides, human cathelicidin LL-37, has an ability to target preformed biofilm. Good activity was reported against biofilms of both Gram-positive and Gram-negative bacteria at one-twentieth of its MIC [79]. Moreover, modified LL-37 peptides showed high efficiencies against biofilm formation by *P. aeruginosa* [80]. Other LL-37 derivatives such as P60.4AC and P10 underwent satisfactory *in vitro* trials against multidrug-

resistant *S. aureus*. Similarly, D-LL-37 was highly active against formation of biofilm and bacterial attachment by *P. aeruginosa* [81][82]. In one successful attempt to control MRSA, applying a cationic peptide lowered MIC values by two-fold [83]. Determining the suitability of each of these products to treat biofilm requires various considerations to be evaluated. From a therapeutic aspect, the extent of any cytotoxic damage should be recognized. AMPs can engender toxicity through pore formation, apoptosis, and necrosis [84].

It is apparent that applying anti-biofilm peptides, either natural or synthetic, has both advantages and disadvantages. The latter include increased manufacturing cost due to the long chain of peptides and complexity, high toxicities, and their susceptibility to host proteases. Modifications performed on peptides can ameliorate these development hurdles. On the other hand, the anti-biofilm activity of AMPs makes them an attractive choice as an alternative treatment. This is especially true if they can boost the efficiency of an antibiotic at a lower dose compared to single antibiotic therapy only [36].

3.8. Other Approaches

There are yet further strategies used to combat biofilm infections, for which major investment is needed to underpin discovery and testing (**Table 1**). A current focus is on repurposing available drugs such as the anti-rheumatic agent auranofin. Several chelators such as ethylenediamine tetraacetic acid, sulfhydryl compounds like dithiothreitol, and phytochemicals extracted from plants, including flavonoids and polyphenolic compounds, are all under investigation [85]. Additionally, UM-C162, a benzimidazole derivative, shows therapeutic promise by interrupting various *S. aureus* virulence factors including hemolysins, clumping factors and proteases [86].

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