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Anti-biofilm peptides as a new weapon in antimicrobial warfare

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Microorganisms growing in a biofilm state are very resilient in the face of treatment by many antimicrobial agents. Biofilm infections are a significant problem in chronic and long-term infections, including those colonizing medical devices and implants. Anti-biofilm peptides represent a very promising approach to treat biofilm-related infections and have an extraordinary ability to interfere with various stages of the biofilm growth mode. Anti-biofilm peptides possess promising broad-spectrum activity in killing both Gram-positive and Gram-negative bacteria in biofilms, show strong synergy with conventional antibiotics, and act by targeting a universal stringent stress response. Understanding downstream processes at the molecular level will help to develop and design peptides with increased activity. Anti-biofilm peptides represent a novel, exciting approach to treating recalcitrant bacterial infections.

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Introduction

Biofilms are multicellular, three-dimensional aggregates that form on surfaces in both nature and the clinic. They are difficult to treat since biofilms are adaptively resistant to antibiotics (up to 1000-fold) as compared to their free-swimming, planktonic counterparts [1]. Biofilms can form on a variety of tissues and implanted devices, and are implicated in diverse diseases such as cystic fibrosis, wounds, otitis media, pneumonia, and osteomyelitis [2]. Bacterial aggregates that form on medical implants, such as catheters, valves, stents and shunts are difficult to remove except by surgery [2]. The annual cost to the U.S. health care system is on the order of billions [3]. Therefore new therapeutic options are urgently needed. The treatment of biofilm-related infections is very challenging and scientific attention has recently turned to developing agents with specific anti-biofilm activity [4,5]. In particular, this review will focus on anti-biofilm peptides, their activity in combination with other antimicrobial agents, and their mechanism of action.

Antimicrobial peptides with potential to fight biofilm-related infections

Antimicrobial peptides (AMPs), characterized here as peptides with activity versus planktonic bacteria, possess broad-spectrum antibiotic activity against most bacterial pathogens. They are a subset of the host defence peptides, named due to their frequent anti-infective immunomodulatory activity, and are an important part of human innate immunity [6]. Importantly, AMPs do not necessarily affect biofilms. For example, numerous peptides have been developed over the past few years, but comparatively few show anti-biofilm activity below their minimal inhibitory concentration (MIC). A few examples of recently described peptides with anti-biofilm properties are shown in Table 1 and described below.

The human cathelicidin peptide LL-37 has very weak AMP (planktonic antibiotic) activity under physiological conditions [7]. A breakthrough was achieved when it was demonstrated that LL-37 inhibited biofilm formation at concentrations 16-fold below its MIC against planktonic bacteria [8•]. LL-37 was subsequently shown to possess anti-biofilm activity against urinary tract isolates of *Staphylococcus aureus* and *Escherichia coli* at 1/32 to 1/2 MIC [9]. Recently, synthetic cathelicidin-derived anti-biofilm peptides (such as innate defense regulator 1018, DJK-5, and DJK-6) were developed, which exhibited broad-spectrum activity against multidrug resistant organisms [10••,11].

Aside from cathelicidins, novel discoveries also draw from the diversity of AMPs found in nearly all domains of life [12]. For example, Anunthawan *et al.* demonstrated that the two tryptophan-rich cationic antimicrobial peptides KT2 and RT2 showed anti-biofilm activity at sub-MIC levels against the multidrug-resistant, enterohemorrhagic *E. coli* O157:H7 strain and were able to prevent biofilm formation and eradicate mature biofilms at a concentration of 1 μM [13]. Both peptides interacted with and bound to negatively-charged LPS molecules to enable self-promoted uptake (without forming pores or aggregates) across the outer membrane and subsequently interacted with cytoplasmic membrane phospholipids [13].

Two classes of peptides with unusual structure were also recently developed. The first, SB056, a semi-synthetic

Table 1

Recent studies on peptides with specific anti-biofilm activity

Peptide	Minimal inhibitory concentration	Active biofilm concentration	Active against	Reference
AS10	50 μM[a]	0.22 μM[b]	*C. albicans*	[16]
KT2 and RT2	5–18 μM	1 μM[c]	*E. coli*	[13]
SB056 and derivatives	10–>40 μM	5–20 μM[d]	*S. epidermidis*, *P. aeruginosa*	[14]
Cyclic lipopeptide **3**	22–55 μM	4 μM[c]	*S. aureus*, *P. aeruginosa*	[15]
LL-37 and derivatives	32 μg/ml	1–16 μg/ml[d]	*S. aureus*, *E. coli*	[9,31]
(IDR-)1018	8–128 μg/ml	2–8 μg/ml[b]	*A. baumannii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. enterica*, *S. aureus*	[10••]
DJK-5	1.6–16 μg/ml	0.8–4 μg/ml[b]	As for IDR-1018	[11]
DJK-6	4–16 μg/ml	0.5–8 μg/ml[b]	As for IDR-1018	[11]

[a] Minimal fungicidal concentration.
[b] Minimal biofilm inhibitory concentration.
[c] Active concentration in flow cells.
[d] Concentrations showing biofilm inhibition.

peptide with a dendrimeric (dimeric) scaffold was active against planktonic *E. coli* and *S. aureus* and showed anti-biofilm activity against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* at concentrations half or less the MICs [14]. Remarkably, an optimized linear form (with enhanced amphiphilic profile) of SB056 as well as the dimeric dendrimer were even more active against *S. epidermidis* biofilms. These peptides showed strong affinity for bacterial membranes and the authors postulated that the distribution of hydrophobic and charged residues within the peptide sequence play a role in peptide–lipid interaction [14]. Secondly, Bionda *et al.* [15] used a positional-scanning combinatorial method to screen a cyclic lipopeptide library (peptides derived from fusaricidin/LI-F) against multidrug resistant pathogens. The lead peptide from this study showed activity against all ESKAPE pathogens at 110 μM. Intriguingly, at much lower concentrations (22–28 μM), antibacterial activity was observed against *Enterococcus faecium*, *S. aureus*, *Acinetobacter baumannii*, and *P. aeurginosa*. Furthermore, at a concentration as low as 4.4 μM, the lead peptide inhibited biofilm formation and eradicated mature biofilms of both *P. aeruginosa* and *S. aureus*. A screen revealed that improved potency depended on hydrophobic as well as positively charged amino acids. These examples highlight the importance of studying and understanding peptide scaffolds, structural order of amino acids within a sequence, peptide activity, and interaction with bacterial membranes.

Peptides can also be active against fungal biofilms. De Brucker *et al.* [16] showed that the cathelicidin-derived peptide AS10 had specific anti-biofilm activity at a concentration of only 0.22 μM (~1 μg/ml) against fungal *Candida albicans* biofilms. This concentration was more than 200-fold less than that needed to inhibit planktonic growth. AS10 also inhibited biofilm formation in a mixed *C. albicans* and *S. epidermidis* population and was active against Gram-negative pathogens including *E. coli*, *P. aeruginosa*, and *Porphyromonas gingivalis*.

Peptides enhance the activity of other antimicrobial agents

In the past few years, many peptides were identified that show strong action against microbial biofilms. Recent studies have also demonstrated that peptides can be used in conjunction with antibiotics, antifungals, or other antimicrobial compounds, which leads to enhanced activity (i.e. synergistic effects) [16–18]. Lowering antibiotic concentrations helps to reduce expenses, toxic side effects, and the spread of antimicrobial resistance. Synergy with peptides can also enhance the activity of antibiotics against multidrug resistant strains [17]. This is highly relevant because biofilm-related infections often result in chronic diseases that fail to be eradicated by antibiotics alone [4].

The synthetic peptides IDR-1018, DJK5, and DJK6 acted synergistically against several Gram-negative pathogens with one or more of the major conventional antibiotics ceftazidime, ciprofloxacin, imipenem and tobramycin [11,19], lowering their effective concentrations up to 64-fold. IDR-1018 also showed synergy with the antiseptic agent chlorhexidine against multispecies oral biofilm [20] and DJK-6 enhanced the activity of the carbapenem imipenem against plasmid-mediated carbapenemase-producing *Klebsiella pneumoniae* [17], highlighting how peptides can be used to repurpose antibiotics.

Not only do peptides show synergy with antibiotics, they also enhance the activity of antifungal drugs. The combination of the lipopeptide bacillomycin D and the antifungal drug amphotericin B was strongly synergistic against *C. albicans* biofilms [18]. Moreover, peptide AS10 was able to act synergistically with the antifungal drugs caspofungin and amphotericin B against *C. albicans* biofilms [16]. The concentration of antifungal required to eradicate a biofilm was reduced 5-fold to 8-fold in the presence of 0.39–1.56 μM AS10 [16].

In addition to their synergy with antimicrobial agents, peptides can also be used to extend the spectrum of