

Antibiofilm Peptide Development for Clinical and Industrial Applications

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Abstract

Natural Antimicrobial peptides (AMPs) are important components of immune systems and possess immuno-modulatory and broad spectrum antimicrobial activities. These host-defense peptides failed to resist protease degradation or to efficiently inhibit biofilm formation. We describe in this review the different strategies to improve AMPs and develop peptides that could be used clinically and in industry through antimicrobial, antibiofilm and/or anti-inflammatory activities, protease resistant property and without being cytotoxic. They can be developed as free molecules or immobilized on surfaces or in synergy with other treatments. AMPs could be the next important step in bacteriology treatment after antibiotic discovery.

Keywords: AMPs, anti-inflammatory, antimicrobial, biofilm, cytotoxic

Introduction

The discovery of antibiotics has revolutionized modern medicine and saved many lives. Nowadays, we know that bacteria are able to develop many strategies to resist against antimicrobial molecules especially antibiotics and the elaboration of new treatments is not only necessary but essential for medical and industrial domains [1-3]. Moreover, microorganisms have capacities to organize in a community lifestyle called a biofilm. Microorganisms are embedded in a matrix composed of polysaccharides, extracellular DNA, proteins, lipids and other components. Biofilm development occurs on biotic surfaces (in wounds or in diseased lungs of cystic fibrosis patients) and on abiotic surface like implants or catheters [4-6]. Medical devices are the perfect *in situ* place for bacteria to adhere (urinary/venous/arterial catheters, respirators, orthopedic prostheses etc. ...). Even tissue fillers used in esthetic purpose could be supports of biofilm infection [7]. About 60-70% of nosocomial infections, which represent the fourth leading cause of death in US are due to biofilm development on medical implants [8]. Bacteria in biofilms are 10 to 1000 times more

resistant to conventional antibiotics than free-floating bacteria according to the strains, the molecule applied and the model of study [4, 9-11]. In consequence, antimicrobial treatments have only an effect on free cells (symptoms could disappear for a while). The infection origin still remains in spite the high doses of antibiotic treatment which lead to the emergence of bacteria super-resistant against different types of antimicrobial agents. The only solution that exists is the removal of the infected material whenever possible, with psychological and physical traumatic consequences [11, 12].

Industrial activities are also concerned by microbial biofilm invasion in pipelines or on metals which leads to biocorrosion. This interferes with many processes like filtration, cooling system or oil and gas extraction in contaminating and in clogging the network. The development of microbial communities on surfaces like ship hulls is named biofouling and are estimated to cost 200\$ billion in the US economy [13, 14].

Biofilm development has a high economical impact in medical and industrial areas, in addition to being responsible for the deaths of many people worldwide (Figure 1).

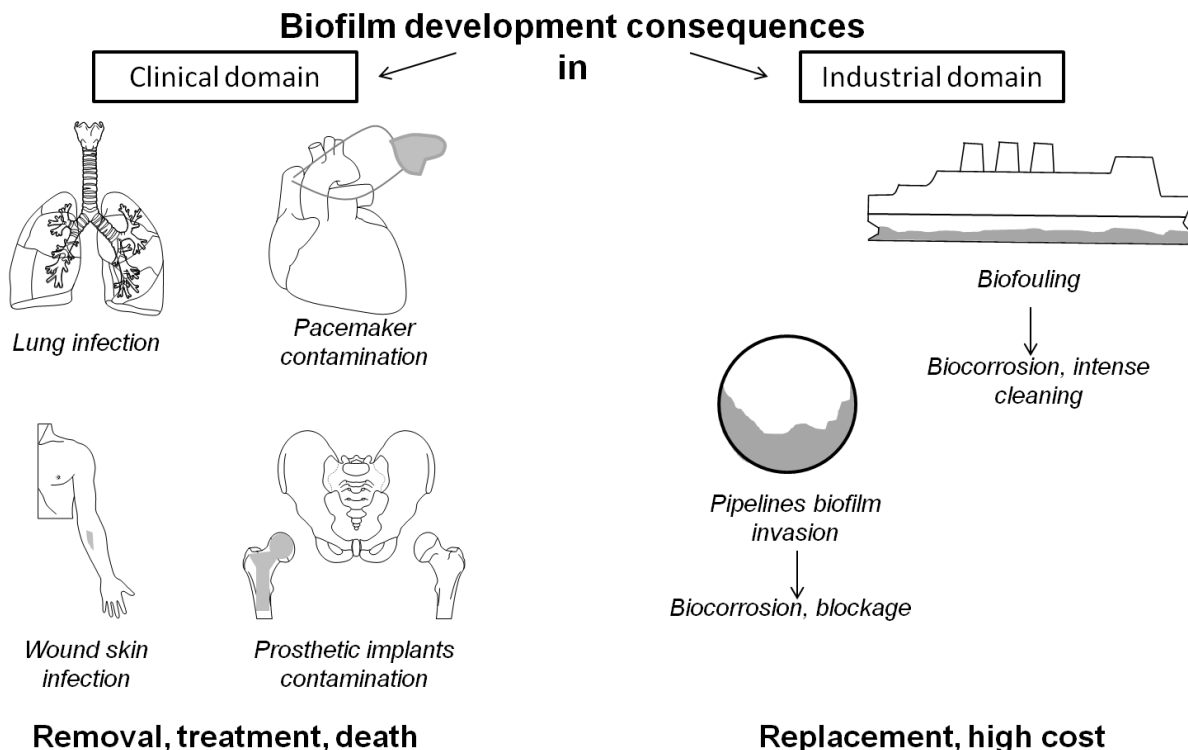


Figure 1: Biofilm development consequences in clinical and industrial domains. Grey shape represents bacterial biofilm formation.

The development of molecules capable of inhibiting biofilm formation and/or efficiently killing mature biofilms is urgent. In clinical settings, there are many goals to reach for eradicating a biofilm infection: to kill planktonic cells, to avoid biofilm development and to deal with the host inflammatory response [4, 6]. Moreover, the use of a molecule requires it to be not cytotoxic and protease resistant.

In consequence, different strategies have been raised to fight biofilm infection based on biofilm development life cycle: adhesion, growth and dispersion [5]. These strategies are prevention (inhibition of adhesion), weakening (slowing growth), disruption (destruction of mature biofilm) and killing [15].

Antimicrobial peptides (AMPs) are an essential part of innate human immunity, present in various organisms (plants, animals, insects, etc.) and possess a broad spectrum activity [16]. Recently, different properties of antimicrobial

peptides (AMPs) have been studied and the design of synthetic sequences has been developed for enhancing antimicrobial, anti-inflammatory or anti-biofilm activities. They represent a potential solution of new treatment in medical or industrial domains [17, 18]. In this review, we describe the strategies aiming the improvement of AMPs to fight against bacterial biofilm and multidrug resistant (MDR) bacteria.

Dual effect development (anti-inflammatory, anti-microbial or anti-biofilm activities)

Many patients suffering from chronic infection have symptoms which lead to suspect the development of a bacterial biofilm, including inflammation, tissue damage, and resistance to both antibiotic and phagocytosis [4, 6]. The presence of biofilm stimulates the host immune response by chemoattractant molecules as *P. aeruginosa* quorum sensing for example.

Immune system cells produce reactive oxygen species, cytotoxic and bactericidal substances, cationic antimicrobial peptides and phagocytic enzymes. Unfortunately, all these molecules do not kill bacteria but lead to high damages in surrounding tissues. Moreover, some free cells dispersing from the biofilm could disseminate, infecting other areas [4, 19].

Improvement of AMPs to obtain dual activity in a single molecule.

Based on those observations, some studies have started to enhance the quality and properties of AMPs in order to make them act with a dual anti-inflammatory and antibiofilm effect or antimicrobial and antibiofilm effects, for example. AMPs are short peptide sequences with high proportion of hydrophobic and positively charged residues. The peptide sequence can be truncated or modified by amino acids substitution to obtain novel sequences. Those designed variants are tested for their activities and then the relationship between structure and activity is studied *in silico* (Figure 2) [20-22].

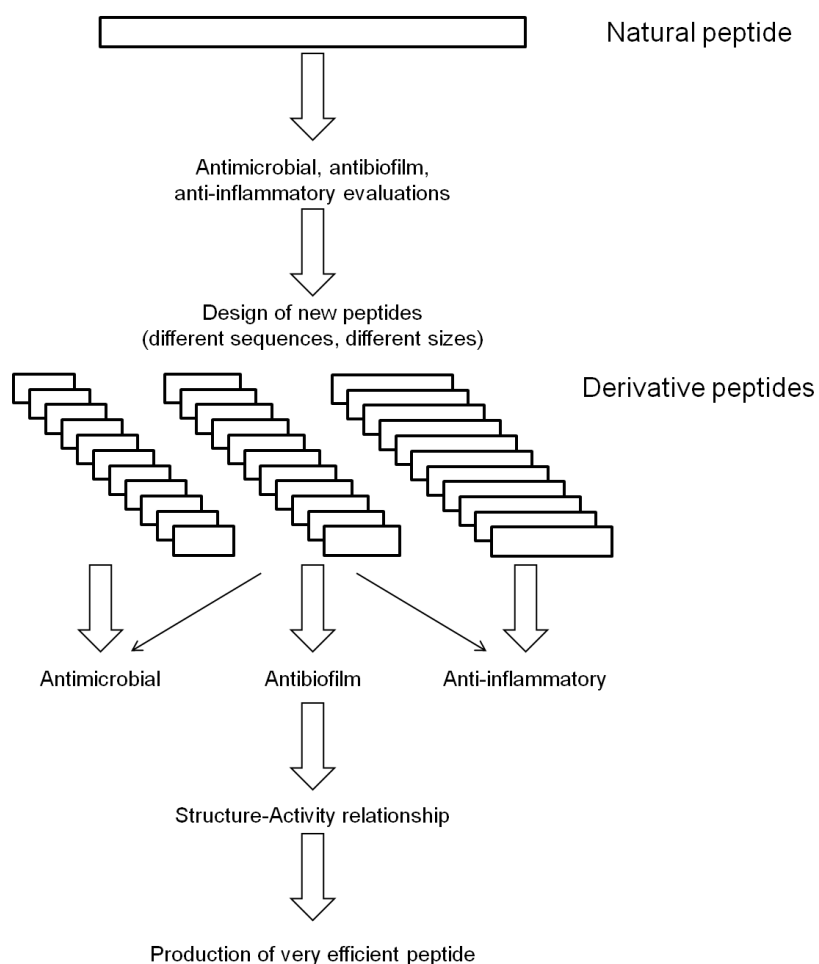


Figure 2: AMPs improvement strategy.

Combination of anti-inflammatory and antibiofilm activities

de la Fuente-Núñez *et al.* selected a synthesized peptide (IDR-1018) based on natural bovine host defense peptide LL37. IDR-1018 targets bacterial

biofilms and also exhibits anti-inflammatory activity [20, 21]. Indeed, immuno-modulatory effects avoid tissue damages and anti-biofilm action inhibits biofilm formation. Another study has shown a screening of hundreds of peptide variants. Using SPOT-synthesized peptide arrays on cellulose membranes, AMPs were selected for their capacity of inhibiting Multi Resistant *Staphylococcus aureus* (MRSA) biofilms and the ability to stimulate production of a monocyte chemoattractant protein (MCP-1) and suppress LPS-induced interleukin (IL)-1 β production in human peripheral blood mononuclear cells (PBMCs). This strategy resulted in the production of second-generation peptides with strong immuno-modulatory and antibiofilm activities [22].

Natural L-amino-acid AMPs are sensitive to enzymatic degradation and synthetic design can be a solution [23]. Thus, improvement of synthesized peptides also leads to production of anti-biofilm D-enantiomeric peptides, which are protease resistant [24]. Those results represent a new way of cure with dual action on host cell and bacteria responses during an infection.

Combination of antimicrobial and antibiofilm activities

Recently two tryptophan-rich antibacterial peptides (KT2 and RT2) showed antimicrobial and antibiofilm effects. This could be interesting in treating acute or chronic infection of both Gram-positive and Gram-negative bacteria inhibiting biofilm formation and killing cells that escape the biofilm and to stop transition to the planktonic lifestyle [25].

Prevention to avoid bacterial adhesion

Prevention is always better than treatment to avoid tissue damage, surgical intervention and economical cost in industry. In consequence, development of strategies to inhibit bacterial adhesion on biotic or abiotic surfaces is interesting to avoid biofilm formation. Different strategies have been studied as immobilization of an AMP on a surface [26]. For example,

gramicidin A was covalently bound to gold surfaces and was able to reduce to 60% *Escherichia coli* attached on material and delayed the biofilm development for at least 24h [27]. AMPs treated surfaces could be one solution to fight against biofouling in industry. This strategy of grafting a peptide could be applied on biomaterial or catheters in clinical perspective. Segev-Zarko *et al.* [28] have suggested that the reduction of bacterial adhesion and biofilm growth was due to their synthesized peptide capacity to coat biomaterial without any surface modification. Urinary tract infection due to catheter is the most common nosocomial infection. Thus, CWR11 a synthetic antimicrobial peptide, was chemically coated on catheter surfaces and displayed broad spectrum antimicrobial and antibiofilm activities for at least 21 days [29]. Coating AMPs on medical devices deliver high treatment doses directly to the targeted infected site [30]. For example, Yoshinari *et al.* [31] observed the reduction of biofilm formation of *P. gingivalis* on a titanium sensor coated with histatin-5 and lactoferricin. In another study, an implant surface carrying nontoxic Tet-20 (based on bovine cathelicidin Bac2A) exhibited broad antimicrobial activities both *in vivo* (rats) and *in vitro* [32].

Instead of being immobilized on a surface, peptides could also be assembled with other molecules, constituting nanomaterials or gel that could be directly applied on wounds responsible for chronic infection [33, 34]. Further, inhibition of bacterial adhesion could be also done releasing AMP from a surface. Multilayer coating of an AMP HHC-36 on titanium surface was effective against both Gram-negative and Gram-positive bacteria without observing cell cytotoxicity [35]. The controlled release of AMP represents a new approach to prevent biofilm formation in clinical and industrial domains.

Synergy between AMPs and other antimicrobial treatments

There are many aspects to deal with during a biofilm infection like dispersion of bacteria that could lead to spread infection or to induce a

septic shock. Antibiotics are efficient against these dispersing bacteria but not low concentrations of AMPs. However, antibiotics are unavailable against adhering cells contrary to some AMPs. Synergy between different molecules allows the combination of their own properties to complete a function like fighting biofilm infection. Peptides could have dual effect but they could also enhance antibiotic activity. Thereby, use of combination between antibiofilm peptide IDR-1018 and sub-minimal concentration inhibition of conventional antibiotics have been shown to inhibit biofilm formation, eradicate established biofilm and reduce live cells dispersion [36]. Four chimeric AMPs tested were effective against 19 *Acinetobacter baumannii* clinical isolates and acted synergistically in combination with cefotaxime, ciprofloxacin, or erythromycin [37]. Also, PMAP-36 and PRW4 show synergy with aminoglycosides (gentamicin) against *E. coli* and *S.aureus* [38]. Those synergistic combinations could be adapted according to the targeted bacterial species. Indeed, colistin plus imipenem treatment is more efficient against *E. coli* and *K. pneumoniae* adhesion whereas colistin plus ciprofloxacin is better to inhibit *P. aeruginosa* biofilm attachment [39]. Furthermore, synergy between α helical AMPs and traditional antibiotics which have been proved *in vitro*, is also revealed an *in vivo* model [40]. There is an abuse of antibiotic use and the control of dosage is difficult. Thanks to the synergistic effect between the peptide and the conventional antibiotics, the concentration of antibiotic is reduced [24, 36]. We can imagine that it would change the view of using antibiotics and reduce the consequences of a highly concentrated treatment. Synergistic action between AMPs and antibiotics are hopefully the future of clinical application in preventing biofilm formation and in eradicating already established biofilm while preventing development of antibiotic or peptide resistance mechanisms.

Efficiency against MDR strains

Recent work has also focused on finding potential treatments against bacterial strains

that are resistant to multiple antibiotics. For example, a number of small cationic peptides have shown capacity to inhibit or eradicate biofilm formed by MDR strains like Methicillin Resistant *Staphylococcus aureus*, *Klebsiella pneumoniae* or *Burkholderia cenocepacia* [20, 25, 41]. Cationic peptides KT2 and RT2 at minimal inhibitory concentrations are capable of killing planktonic multidrug resistant bacteria, enterohemorrhagic pathogen *E. coli* O157:H7. At sub-MIC concentrations, these peptides have the capacity to prevent biofilm formation and even trigger killing of cells from mature biofilm of *E. coli* O157:H7. KT2 and RT2 could be used against multidrug resistant *E. coli* strain during both accurate and chronic infection [25]. Burn wounds are often infected by MDR strains like *S. aureus*. LL37-derivative peptides developed by Haisma *et al.* (2014) eradicate mupirocin-resistant MRSA strains and are attractive candidates of new local therapy [41]. Action of AMPs is probably excluded of the diverse mechanisms inducing antimicrobial resistance. In consequence, AMPs represent an important strategy to develop against MDR strain infections, especially to treat superbugs that could develop biofilm, in clinical or industrial domains.

Discussion

AMPs-based therapeutics should be considered as a very promising future option against the increase of bacterial antibiotic resistance and the decrease of introduction of new antibiotics [42]. Many benefits in clinical and industrial domains are possible thanks to the improvement of antimicrobial peptides. Designed synthetic AMPs are optimized for their antimicrobial, anti-biofilm activities while minimizing toxicity and production costs [42]. At first, AMPs possess combined actions such as antimicrobial and antibiofilm or anti-inflammatory and antibiofilm properties [20-22, 25]. Moreover, they have broad-spectrum effects, even against MDR strains [20, 25, 41] and their use at low rate avoids the induction of bacterial resistance [30]. Some of them have shown a potential synergy with other antimicrobials [36-38]. Thus a peptide

with both anti-inflammatory and anti-biofilm activities combined with antibiotics could eliminate free bacteria and biofilm during a chronic infection and that without excessive inflammatory response. In eliminating biofilms, the requirements are to minimize dispersal of live cells and prevent induction of septic shock. Synergy between AMPs and antibiotics could be useful to prevent this risk. Moreover, high-dose treatment that does not kill biofilm cells can induce bacterial resistance. The combination of low concentration of both AMPs and antibiotics would eradicate biofilm infection while avoiding exposition to sub-lethal concentration and so the emergence of resistant superbugs.

Importantly, these peptides are found to be mostly non-cytotoxic. Peptide improvement research as the creation of protease-resistant molecules gives much hope for the future of AMPs in clinical and industrial applications [20, 23]. Moreover, if the peptide sequence is reduced to the optimal size, the production cost will be much lower.

Much remains to be studied, but these peptides have many possible applications. In medical treatment, AMPs fixed on implant surfaces could prevent infections due to the insertion of foreign material in the body. The production of gel containing AMPs could be used to treat infected wounds as burn wounds which are infection sensitive sites and could accelerate healing through anti-inflammatory action [21, 34, 41].

In the industrial field, pipelines used in various processes treated with free or immobilized AMPs would save billions of dollars by preventing biocorrosion. Also, the use of AMPs in the cosmetic and pharmaceutical industries allows the creation of high quality bacteria-free products.

Some challenges are still remaining to perfect these AMPs. For example, small numbers of companies are researching on AMPs as

therapeutics [42] and none or only a few of these molecules pass clinical assays. Immobilization of all AMPs is not possible as they possess different mechanisms of action [30]. The main problem of antibiotics is the ability of bacteria to develop strategies to counter its effects. Use of lower concentrations of peptide could avoid the set up of resistance mechanisms. However, some biofilm resistance mechanisms against AMPs were already described. Some components of the matrix like exopolymers or alginate could interact and sequester AMPs [43, 44]. Extracellular DNA (eDNA) could also bind to AMPs [45]. Synergism between molecules is interesting but only if this association does not imply a cross-resistance. An example of inefficient treatment is the use of LL-37 and polymyxin B which induces a modification of a matrix protein and leads to the protection of *Vibrio cholerae* from LL37 treatment [46]. Moreover, the production of synthesized peptides could be problematic and expensive. The process of production (chemical way or through a heterologous microbial system) and the purification can be complicated by the nature and the structure of the peptides [47]. For exploitation of many peptide variants, standardized operating protocols for anti-biofilm test and automated tools for screening will be useful [48].

Conclusion

Antimicrobial peptides are a promising therapeutic option for antimicrobial and antibiofilm treatments in clinical or industrial fields. AMPs could eliminate planktonic bacteria, inhibit biofilm formation and eradicate mature biofilms in a broad-spectrum manner (AMPs properties are summarized in Figure 3). Moreover, they can be easily modified to enhance their activity.

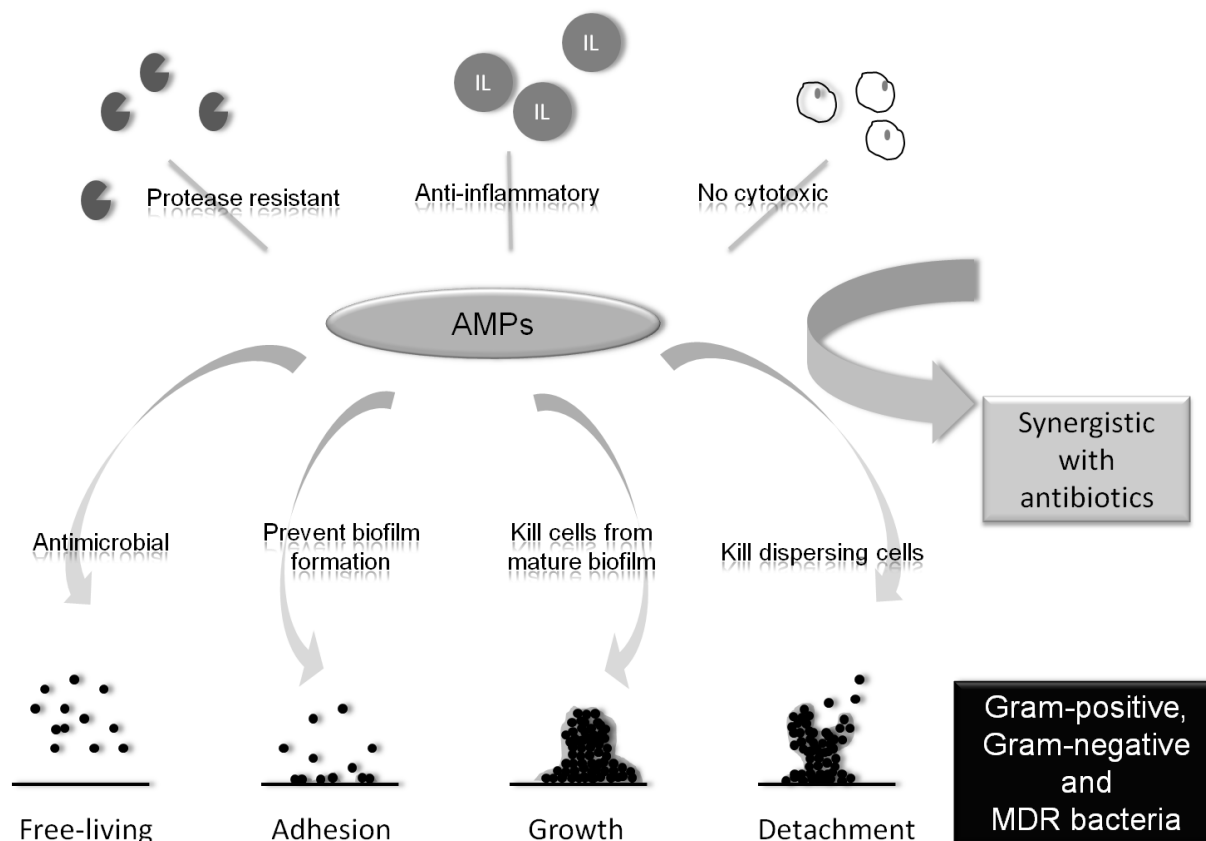


Figure 3: AMPs properties. Schematic representation of biofilm development, AMPs properties and how they can affect different stages of biofilm formation (IL = Interleukin).

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