Published in final edited form as:
Int J Antimicrob Agents. 2011 September ; 38(3): 217–225. doi:10.1016/j.ijantimicag.2011.05.004.

Will new generations of modified antimicrobial peptides improve their potential as pharmaceuticals?

Nicole K. Brogden[a] and **Kim A. Brogden**[b,*]

[a]Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536, USA

[b]Department of Periodontics and Dows Institute for Dental Research, N447 DSB, College of Dentistry, The University of Iowa, 801 Newton Road, Iowa City, IA 52242, USA

Abstract

The concept of antimicrobial peptides (AMPs) as potent pharmaceuticals is firmly established in the literature, and most research articles on this topic conclude by stating that AMPs represent promising therapeutic agents against bacterial and fungal agents. Indeed, early research in this field showed that AMPs were diverse in nature, had high activities with low minimal inhibitory concentrations, had broad spectrums of activity against bacterial, fungal and viral pathogens, and could easily be manipulated to alter their specificities, reduce their cytotoxicities and increase their antimicrobial activities. Unfortunately, commercial development of these peptides, for even the simplest of applications, has been very limited. With some peptides there are obstacles with their manufacture, in vivo efficacy and in vivo retention. More recently, the focus has shifted. Contemporary research now uses a more sophisticated approach to develop AMPs that surmount many of these prior obstacles. AMP mimetics, hybrid AMPs, AMP congeners, cyclotides and stabilised AMPs, AMP conjugates and immobilised AMPs have all emerged with selective or 'targeted' antimicrobial activities, improved retention, or unique abilities that allow them to bind to medical or industrial surfaces. These groups of new peptides have creative medical and industrial application potentials to treat antibiotic-resistant bacterial infections and septic shock, to preserve food or to sanitise surfaces both in vitro and in vivo.

Keywords

Antimicrobial peptide mimotopes; Hybrid antimicrobial peptides; Antimicrobial peptide congeners; Stabilised antimicrobial peptides; Antimicrobial peptide conjugates; Immobilised antimicrobial peptides; Cyclotides

*Corresponding author. Tel.: +1 319 335 8077; fax: +1 319 335 8895. kim-brogden@uiowa.edu (K.A. Brogden).

Competing interests
None declared.

Ethical approval
Not required.

1. Introduction

As their name implies, antimicrobial peptides (AMPs) are small molecules with antimicrobial activity, despite wide variations in their mass, amino acid residue composition, charge, three-dimensional structure and biological characteristics. They are now known to be a vast group of molecules widely distributed throughout nature and produced in species of the kingdoms Monera (e.g. Eubacteria), Protista (e.g. protozoans and algae), Fungi (yeasts), Plantae (plants) and Animalia (e.g. insects, fish, amphibians, reptiles, birds and mammals) [1]. In some species these peptides serve as the primary antimicrobial defence mechanism, yet in other species they serve as an adjunct to existing innate and adaptive immune systems.

Interest in AMPs as potential antibiotic pharmaceuticals has always been high [2]. Because of their rapid and broad-spectrum antimicrobial properties, these peptides were quickly proposed as antimicrobials to treat microbial infections, particularly those caused by antibiotic-resistant bacteria. Current research is divided into many areas. One area continues to focus on identifying the spectrum of AMPs in nature, determining their range of antimicrobial activities against bacteria, fungi and viruses, identifying their mechanisms of antimicrobial activity in model membrane systems, identifying their mechanisms of antimicrobial activity in intact microorganisms, and assessing their cytotoxicity to eukaryotic cells and erythrocytes. A second area of research focuses on the role of AMPs in innate immunity, their ability to attenuate the induction of pro-inflammatory cytokines and their role in adaptive immune mechanisms [3,4]. This is particularly true for defensins, which have direct antimicrobial activity, chemoattract phagocytic and mast cells, induce inflammatory mediators, regulate the functions of phagocytes, and regulate the complement system [5,6]. A third area of research, and the focus of this review, is the development of modified peptides with unique properties. This includes AMP mimetics, hybrid AMPs, AMP congeners, stabilised AMPs, AMP conjugates and immobilised AMPs.

It is generally accepted by many investigators that naturally occurring AMPs per se may not be suitable for pharmaceutical development. In fact, commercial development of these peptides for even the simplest of applications has been very limited. However, there are some AMP compounds that have reached clinical trials [7,8]. The stages of development of additional trials for immunomodulatory anti-infectives with antimicrobial actions, immunomodulatory anti-infective peptides lacking antimicrobial action, immunomodulatory peptides, and anti-infective peptides with unknown immunomodulatory activity are listed in Table 3 of the recent article by Steinstraesser et al. [8]. These compounds have been examined as active treatments for a variety of medical uses, including diabetic foot ulcers, prevention of catheter-related bloodstream infections, therapy of acute acne, etc. The discovery, preclinical status, phase status and post status of many of these compounds in clinical trials can be easily searched and cross-checked (http://clinicaltrials.gov/).

Despite the clear potential of these compounds and the extensive efforts to move them into a clinical realm, the resulting success has been limited. One nearly successful peptide in clinical trials was MSI-78 (pexiganan acetate). This topically administered peptide reached phase III clinical trials and was found to be as effective as the oral antibiotic ofloxacin in the treatment of diabetic foot ulcers. Unfortunately, the new drug application was ultimately not approved by the US Food and Drug Administration (FDA) [7,9,10]. Omiganan (MX-226 or CPI-226) represents another near success story, as this peptide missed its primary endpoint in phase 3a clinical trials for preventing catheter-associated infections. Omiganan did achieve significance in other endpoints (reducing catheter colonisation), allowing it to progress into confirmatory 3b trials.

The near success of these compounds still helps to maintain an enthusiastic attitude amongst pharmaceutical companies and pre-clinical researchers about their potential for commercial development. However, there are still a number of roadblocks that must be addressed before clinical implementation of AMPs will be attainable. Most notable are the disadvantages in the production, properties and efficacy of AMPs. Potentially the largest issue in the field is the projected high manufacturing cost of these peptides. If these agents will ever truly move forward into clinical practice, a less costly means of production must be developed. Recombinant DNA methods have been explored to generate the compounds, using production systems in a variety of organisms (bacteria, fungi, plant and animal systems have all been explored); in general, however, these methods have failed to prove commercial feasibility. The pharmacokinetic properties of the AMPs can be somewhat unfavourable, as these peptides are highly susceptible to degradation by proteases and exhibit short half-lives. Lastly, AMPs have had low bacteriological efficacy in animal models, and the loss of activity under physiological conditions is a valid concern with the general applicability of this entire class of compounds.

Regardless of the challenges noted above with developing AMPs for clinical use, the rise of resistant bacteria has prompted the continual search for new AMPs and this interest has never waned. The purpose of this review is to describe the newer functional classes of modified AMPs that have the potential to overcome these hurdles and become an important class of available antibiotics. Here we present some of the contemporary strategies proposed to design and engineer AMPs for unique and specific applications. These applications are not all designed solely for preventing or treating microbial infections in humans or animals, but have expanded applications as slow delivery systems, wound dressings, food preservation systems, and coatings for implants, catheters and toys. Even when immobilised or in complex environments, AMPs still retain their antimicrobial activity, which questions earlier work on their mechanisms of action, particularly for AMPs known to form well structured pores. The descriptions, potential and mechanisms of action of these new groups of modified AMPs have served as the topics of many excellent and recent reviews (Table 1).

2. Rational design of modified antimicrobial peptides

By their nature, AMPs are diverse. They have different lengths, amino acid sequences, anionic or cationic charges, and sometimes different tertiary structures [30]. Despite this diversity, they do have some common features in that they are charged and their active regions are generally amphipathic [31]. The designs of modified AMPs utilise these common traits. As pointed out by Tossi et al. [31], the design of AMPs is generally based on (i) amino acid residue analogues of natural peptides (e.g. congeners) that differ at one or more residue positions, are shortened or contain deletions, as well as hybrid AMPs composed of fragments of two different natural peptides; (ii) amino acid residues that maximise the amphipathic nature of AMPs; (iii) amino acid sequences from combinatorial libraries; and (iv) amino acid sequences that are patterned from known, naturally occurring, α-helical peptide domains.

Rational designs also need to overcome some of the shortcomings of AMPs. Consideration has to be given to control production costs, antimicrobial activity in complex environments, the appropriate range of antimicrobial activity, and the host response to the peptide. Modified peptides must contain the smallest amino acid domain that has full antimicrobial activity, one approach that reduces costs for commercial development. Modified peptides must retain antimicrobial activity in adverse environments, a second approach that maintains or increases their activity in complex biological fluids, serum and high physiological salt concentrations. Finally, modified peptides must resist degradation by host enzymes or have a narrower spectrum of activity, a third approach to enhance specific antimicrobial activity

against a select microorganism. These approaches are varied and have resulted in a number of distinctive classes.

3. Modified antimicrobial peptides

Molecules imitating AMP structure and function, or modifications to AMP composition have resulted in a number of distinctive AMP classes that now contain AMP mimetics, hybrid AMPs, AMP congeners, stabilised AMPs, AMP conjugates and immobilised AMPs [12,13].

3.1. Antimicrobial peptide mimetics

Mimetics are synthetic, non-peptidic molecules that mimic the properties and activities of naturally occurring AMPs. AMP mimetics are constructed from peptoids, β-peptides, arylamides, oligomers or phenylene ethynylenes (Table 2). These molecules are designed to capture the central physicochemical features of their natural AMP archetypes thereby mimicking peptide activity and function [40,41].

Peptoids are short, linear peptides with hydrophobic tails like fatty acids that are not normally acylated. Peptoid synthesis incorporates an alkylamine into the peptoid as an N-terminal alkyl tail. These tails can range from 5 to 13 carbons long. Peptoids of various compositions and sizes have differing antibacterial, antifungal and haemolytic activities [32]. A peptoid of only five monomers has selective broad-spectrum antimicrobial activity with potency similar to that dodecameric peptoids [32]. A tetrameric, alkylated, cationic peptoid (1-$C13_{4mer}$) is active against *Mycobacterium bovis* bacille Calmette–Guérin (BCG) and *Mycobacterium tuberculosis* [33], and peptoid 1 and 1-$C13_{4mer}$ are active against *Pseudomonas aeruginosa* biofilms [34].

Mimetics based on acyl-lysine oligomers are being assessed by Amram Mor's laboratory and also have antimicrobial activity [35] and novel antitumour activity (Table 2). Oligo-acyl-lysine (OAK) molecules are composed of tandem repeats of acyl-lysine molecules. Depending upon their composition, OAKs exert potent antimicrobial activity against Gram-positive bacteria while exhibiting negligible haemolysis [36]. However, activity may be reduced by decreases in temperature and pH or increases in ionic strength. For example, $C_{12}K\text{-}7\alpha_8$ activity for *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* is decreased at acidic pH, low temperatures or in high salt concentrations, but activity is enhanced at basic pH or higher temperatures [42]. More complex mimetics, such as N_{α}-hexadecanoyl-$_L$-lysyl-$_L$-lysyl-aminododecanoyl-$_L$-lysyl-amide ($c_{16}KKc_{12}K$) or hexadecenoyl-$KKc_{12}K$ [$c_{16(\omega 7)}KKc_{12}K$] form fibres or nanofibre networks with distinct binding properties to phospholipid membranes [43].

Mimetics based on phenylalkyne and arylamide compounds have antibacterial, antifungal and anti-inflammatory activities [37–39] (Table 2). In the oral cavity, AMP mimetics have the potential to eliminate periodontopathogens. Phenylalkyne compounds, arylamide compounds and the mimetic designated mPE have potent antifungal activity against planktonic cultures and biofilms of *Candida* spp. [37] as well as antimicrobial activity against biofilms of both *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* [38].

3.2. Hybrid antimicrobial peptides

Hybrid AMPs are constructed of the active regions of two to three naturally occurring peptides to capture and combine the potential benefits of each individual fragment. Examples of hybrid AMPs are shown in Table 3. The goals are to increase antimicrobial activity, reduce the antimicrobial spectrum of activity, and reduce cytotoxicity for host cells.

These peptides can be constructed by direct chemical synthesis of the desired hybrid peptide or produced as a fusion protein expressed in an appropriate recombinant system.

The family of cecropin A–melittin (CEME), CEMA, CP26 and CP29 family of peptides produced by Robert Hancock's laboratory are good examples of the hybrid peptide concept [44–48]. CEME is composed of amino acid residues 1–8 of cecropin A and amino acid residues 1–18 of melittin; CEMA is a congener of CEME with a modified C-terminus containing two additional cationic charges; CP26 is a congener with additional cationic charges; and CP29 is a peptide with increased α-helical content. These peptides have an improved ability to permeabilise the membrane of *P. aeruginosa* and can bind to lipopolysaccharide (LPS) and lipoteichoic acid (LTA) [44]. As an added benefit, CEME, CEMA and CP29 retain antimicrobial activity in a physiological range of sodium chloride concentrations [44]. Another hybrid composed of amino acid residues 1–8 of cecropin A and amino acid residues 1–12 of magainin Z has high antitumour activity, low haemolytic activity and induces release of vesicle-entrapped fluorescence probes [55]. On a practical side, expression of CEME and CEMA in transgenic plants may likely confer resistance to bacterial and fungal phytopathogens [56,57].

Hybrid peptides can be designed to have very narrow microbial target specificities. This is achieved by attaching a targeting peptide domain (e.g. a pheromone-like domain) to a membrane-penetrating or intracellular-killing domain. Staphylococcal AgrD1 pheromone attached to colicin Ia kills *S. aureus* but not *Staphylococcus epidermidis* or *Streptococcus pneumoniae* [51]; enterococcal cCF109 pheromone attached to colicin Ia kills vancomycin-resistant *Enterococcus faecalis* [52]; and *Streptococcus mutans* competence stimulating peptide attached to an AMP domain kills *S. mutans* [53]. In some cases, multiple targeting domains can be combined, resulting in a multitargeted peptide [54]. If the domains have different modes of killing (e.g. dual modes of action) this increases the potency, and one such peptide (A3-APO) can disintegrate bacterial membranes and inhibit the 70-kDa heat shock protein DnaK [49,50].

As we have found in our work, not all hybrid AMPs meet expectations. Peptide PQGPPQ from proline-rich protein 1 has an affinity for fimbriae of *P. gingivalis*, and attaching it to either the N-terminus or C-terminus of SMAP28 does not greatly increase the antimicrobial activity or specificity of SMAP28 for *P. gingivalis* [58].

3.3. Antimicrobial peptide congeners

A congener is a chemical compound closely related to another in composition, exerting either similar or antagonistic effects. AMP congeners are prepared by (i) relaxing their tertiary structure when present, (ii) 'swapping out' specific amino acid residues within the parent AMP sequence to change the charge and/or amphipathic characteristics of the molecule, (iii) systematically truncating the N-terminus and/or the C-terminus ends of the parent peptide to identify the smallest size of the active domain or (iv) both 'swapping out' specific amino acid residues and simultaneously truncating either or both the N-terminus or the C-terminus ends of the parent peptide. Many effective congeners of defensins, CAP18, human CAP18/LL-37, SMAP29 and enterocin have been found using this approach (Table 4).

One very effective method of preparing congeners is relaxing their structure. β-Defensins are characterised by three intramolecular disulfide bonds contributing to a defined tertiary structure [80,81]. Reducing the disulfide bonds in human β-defensin-1 (HBD1) increases its antimicrobial activity against *Candida albicans* and against anaerobic *Bifidobacterium* and *Lactobacillus* spp. [82]. Free cysteines in the C-terminus are important for the bactericidal

effect. Also, congeners of linear β-defensins have differing chemotactic activities related to their conformational status [83].

Disulfide bonding in HBD3 is also necessary for binding and activation of cellular receptors for chemotaxis but not for antimicrobial activity [84–88]. Reordering disulfide bond linkages, substituting the cysteine amino acids with α-aminobutyric acid or other amino acid residues, or removing the disulfide bonds completely abolishes the chemotactic activity and diminishes the cytotoxicity of the HBD3 congener but does not alter its antimicrobial activity.

Distinct regions within β-defensins appear to be related to both the spectrum and potency of antimicrobial activity [89–91]. In the murine β-defensin Defb14, congeners of the N-terminus have potent antimicrobial activity for Gram-negative bacteria, and congeners of the C-terminus have poor antimicrobial activity against Gram-negative and Gram-positive bacteria [91]. In HBD1 and HBD3, the internal regions of HBD1 and the C-terminal region of HBD3 are critical for antibacterial activity at high salt concentrations [90]. Deletion of the N-terminal region of HBD3 results in an increase in antibacterial activity. HBD1 and HBD3 congeners inhibit herpes simplex virus and are chemotactic for granulocytes and monocytes.

Linear HBD3 fragments have antibacterial and antifungal activities and decreased cytotoxicity for human conjunctival epithelial cells [87,92,93]. A C-terminal (R36-K45) analogue peptide Y2, with the two cysteine residues replaced by tyrosines, has higher antibacterial activity against *P. aeruginosa* and lower cytotoxicity against mammalian cells than the parent HBD3 [92].

The approach of making congeners also works well with bacteriocins. Enterocin CRL35 is a 43-amino acid residue peptide with activity against *Listeria* spp. [94]. A shorter 15-amino acid residue congener derived from enterocin CRL35 also inhibits the growth of *Listeria innocua* [minimal inhibitory concentration (MIC) = 10 μM] and *L. monocytogenes* (MIC = 50 μM). Similarly, 15-amino acid residue congeners derived from mesentericin Y105, pediocin PA-1 and piscicolin 126 also have antimicrobial activities.

Congeners also can bind to LPS, a major constituent of the outer membrane of Gram-negative bacteria that is highly toxic and can cause sepsis or septic shock. Congeners of the LPS-binding peptide Li5-001 had an ca. 1000-fold higher affinity than Li5-001 and a Kd value of 0.01 nM [95].

Congeners can have novel applications. Arginine-rich peptides can act as potential carriers for intracellular protein delivery [96]. Histidine-rich AMPs can complex with DNA for utility in gene transfer [97]. A Gly-Ala substituted magainin can increase skin permeability and may facilitate transdermal drug delivery [98].

Other modifications in AMPs increase their antimicrobial activities. End-tagging of kininogen with hydrophobic amino acids, for example, increases antimicrobial activity with tag length and leads to more pronounced *P. aeruginosa* LPS binding at high electrolyte concentration and in the presence of plasma or anionic macromolecular scavengers [99].

How small can congeners be? Three amino acid residues appears to be the minimal peptidic sequence for antimicrobial or anti-inflammatory activities of some peptides. The minimal antimicrobial sequence for haemoglobin α-chain is KYR [100] and the minimal sequence for the anti-inflammatory activities of salivary gland prohormone SMR1 is FEG [101].

3.4. Cyclotides and stabilised antimicrobial peptides

Cyclotides are cyclopeptides with a head-to-tail cyclic backbone, containing ca. 30 amino acid residues with three conserved disulfide bonds [22–25]. The bonds form a cysteine 'knot' where the cys1–cys4 disulfide bond and the cys2–cys5 disulfide bond form a ring with their interconnecting backbone segments that is penetrated by the cys3–cys6 disulfide bond. Although only 140 cyclotides have been sequenced from plants in the Rubiaceae (coffee), Violaceae (violet), Fabaceae (peas and beans) and Cucurbitaceae (cucumbers, squashes and pumpkins) families, it is predicted that over 50 000 cyclotides exist in plants from alpine, desert, rainforest and urban environments.

Cyclotides have promising potential pharmaceutical and agricultural applications as anti-HIV, antimicrobial, cytotoxic anticancer, insecticidal, molluscicidal and nematicidal agents. Kalata B1, circulin A, circulin B and cyclopsychotride A have antifungal activity against *Candida kefyr* and *Candida tropicalis* and antibacterial activity against *E. coli*, *Klebsiella oxytoca*, *P. aeruginosa*, *Proteus vulgaris* and *S. aureus* [102]. They are very amenable to chemical modifications, suggesting they be used as scaffolds for peptide-based drug design [23].

Cyclotides are very stable by virtue of their cyclic conformation and their cysteine knot. They are resistant to heat and to degradation by proteolytic enzymes. Likewise, other AMPs can be cyclised and made more active and more stable in adverse environments. Circularised peptide analogues of rabbit defensin NP-1 retain antimicrobial activity in low salt concentrations, have greatly improved antimicrobial activity in higher salt concentrations (100 mM), have less haemolytic activity for human erythrocytes, and have less cytotoxic activity for human cervical epithelial cells [103]. Circularised peptide analogues of enkephalin, contryphan, inhibitors of human tripeptidyl peptidase II, and inhibitors of spider venom epimerase are more stable and resistant to protease degradation [104]. Cyclised angiotensin is fully resistant against purified angiotensin-converting enzyme, has significantly increased stability in homogenates of different organs and in plasma derived from pig, induces relaxation of precontracted Sprague–Dawley rat aorta rings in vitro, interacts with the angiotensin receptor, and displays a strongly (34-fold) enhanced survival in Sprague–Dawley rats in vivo [105]. A cyclic diastereomeric lysine ring analogue of gramicidin S exhibits enhanced antimicrobial activity and markedly reduced haemolytic activity compared with gramicidin S itself [106].

3.5. Antimicrobial peptide conjugates

If AMPs were coupled to a specific outer surface antibody or a ligand for a receptor on a specific bacterial pathogen, they could be used at lower concentrations, would have narrow-spectrum or 'targeted' antimicrobial activities, and induce fewer side effects. There are a few examples that this concept works (Table 5). In plants, coupling antifungal peptides to a *Fusarium* spp.-specific antibody protects plants against a *Fusarium oxysporum* f. sp. *matthiolae* infection [107]. In the oral cavity, we have shown that conjugated peptides have the potential to eliminate a specific periodontopathogen, such as *P. gingivalis*, from patients with periodontal disease without harming the normal commensal flora. Coupling an antibody specific to the outer surface of *P. gingivalis* strain 381 to SMAP28 selectively kills *P. gingivalis* in an artificially generated microbial community containing *P. gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Peptostreptococcus micros* [108]. Furthermore, a disubstituted dexamethasone–spermine conjugate kills *S. aureus* and *P. aeruginosa*, enhances *S. aureus* susceptibility to antibiotics, and inhibits interleukin (IL)-6 and IL-8 release from LPS- or LTA-treated neutrophils [112].

Peptides can be conjugated to micelles and liposomes equipped with uptake-mediating mechanisms. Apolipoprotein E-derived peptide efficiently translocates across cell membranes and integrates into lipid bilayers [109], and this peptide in micelles (colloidal P2A2 micelles) or liposomes (P2A2-tagged liposomes) is taken up differently by endothelial cells of small capillaries (b.End3 cells) and large vascular vessels (BAEC cells). P2A2-tagged liposomes are non-selectively internalised into both b.End3 and BAEC cells via clathrin- and caveolin-independent endocytosis. In contrast, colloidal P2A2 micelles only enter b.End3 cells via clathrin-mediated endocytosis, and not BAEC cells.

Conjugation of AMPs with fatty acids or steroids increases antimicrobial activity. Lactoferrin/lauric acid, peptide AKK/lauric acid, peptide LKK/lauric acid, peptide AKK/myristic acid and VKF, VKW or FFK/triamino analogue of cholic acid conjugates all have increased antimicrobial activity [113,114].

AMPs are powerful adjuvants enhancing immune responses in conjugates containing both AMPs and antigens or as fusion proteins containing expressed AMPs and antigens. LL-37 enhances an antitumour immune response of M-CSFR$_{J6-1}$, a potential target for tumour immunotherapy, when the two are genetically fused and administered to mice [110]. Anti-M-CSFR antibody or M-CSF soluble receptor inhibits the growth of leukaemia and hepatoma cell lines overexpressing M-CSF and M-CSFR. Mouse β-defensin-based vaccines elicit potent cell-mediated responses and antitumour immunity when genetically fused with another non-immunogenic tumour antigen [111]. The fusion protein, consisting of mouse β-defensin linked to a tumour antigen, acts directly on immature dendritic cells as an endogenous ligand for TLR4 and upregulates co-stimulatory molecules, induces dendritic cell maturation and induces the production of lymphokines.

3.6. Immobilised antimicrobial peptides

AMPs and proteins can be immobilised via incorporation into a variety of materials or adsorbed to a variety of surfaces where they still retain their ability to bind and kill bacteria. Both of these methods of immobilisation have vast applications (Table 6) (see Costa et al. [28] for a comprehensive review of this topic).

Native peptides in solution interact with and disrupt microbial membranes with high affinity and well described mechanisms [130]. Immobilised peptides appear to interact and disrupt microbial membranes in a similar fashion, provided they are tethered properly [118]. Still, there can be some reduction of activity. AMPs immobilised close to the surface are thought to electrostatically attract adjacent bacteria, allowing the peptide to penetrate into the membrane of attached bacteria [115].

AMPs can be incorporated into plastics or films to retard spoilage and increase food preservation times. In polyethylene film polymers, nisin inhibits the growth of *Brochothrix thermosphacta*, a psychrotrophic meat spoilage microorganism, on beef surfaces at 4 °C for up to 21 days [116]; in polyelectrolyte multilayer films, an *Anopheles gambiae* mosquito defensin reduces the growth of *E. coli* by 78.6% and *Micrococcus luteus* by 86.4% in growth assays [117]; and in polyelectrolyte multilayer films on silicon wafers, gramicidin A both prevents the growth of *E. faecalis* and lyses the microbial cells that do attach [115].

AMPs can also be tethered to resins or brush layers with proposed uses as contact-active cationic antimicrobial surfaces [118,119]. Peptide KLAL and magainin-derived peptide MK5E immobilised to resins has antimicrobial activity for *E. coli* and *Bacillus subtilis* [118]. Immobilisation reduces the antimicrobial activity but not the spectrum of activity. Longer spacers between the resin surface and peptides KLAL and MK5E and the chain position of immobilisation are more import to antimicrobial activity than surface density of

the peptides. As an antibacterial coating, magainin I in 2-(2-methoxyethoxy) ethyl methacrylate and hydroxyl-terminated oligo(ethylene glycol) methacrylate retains antibacterial activity against the food-borne disease-inducing microorganisms *Listeria ivanovii* and *Bacillus cereus* [120]. Again, tethering is important and the amount of available magainin I can be increased by varying the composition of the copolymer coating.

AMPs can be directly adsorbed to surfaces, also with proposed uses as contact-active cationic antimicrobial surfaces. Nisin adsorbed to stainless steel, polyethylene terephthalate and rubber surfaces inhibits the growth of *Enterococcus hirae*, whilst nisaplin, a congener of nisin, adsorbed to surfaces reduces the attachment of *L. monocytogenes* [123]. Microbial counts of skim milk in nisin-adsorbed PET bottles are significantly lower after 24 days of refrigerated storage.

AMPs and lysozyme can be encapsulated within silica or titania nanoparticles to create bionanocomposite materials with antimicrobial activity for use as broad-spectrum antifouling materials or cosmetics with antimicrobial and sunscreen properties [125]. Lysozyme catalyses the precipitation of silica from tetramethoxysilane to form interconnected nanospheres entrapping the biologically active lysozyme. LL-37 in silica precipitated from tetraethyl orthosilicate is slowly released and has high antimicrobial activity against *E. coli* and *S. aureus*, low haemolytic activity for erythrocytes, and low cytotoxicity against keratinocytes [126].

Immobilised AMPs can be used to capture, concentrate and detect microorganisms in screening assays for the detection of food-borne pathogens or microbial biothreat agents. Cecropin A, magainin I and parasin immobilised on silanised glass slides with bifunctional N-(γ-maleimidobutyryloxy) succinimide ester spacers binds *E. coli* O157:H7 and *Salmonella enterica* serovar Typhimurium [127,128]. Detection limits in sandwich assays were $0.5–5.0 \times 10^5$ colony-forming units (CFU)/mL (*E. coli*) and $0.1–5.0 \times 10^6$ CFU/mL (*Salmonella* Typhimurium). This technology has the application to detect bacterial, viral and rickettsial pathogens, including inactivated biothreat agents [129].

In the oral cavity, peptides immobilised to denture material may serve to prevent biofilm formation, and peptides immobilised to titanium surfaces may serve to shorten the period of osseointegration of implants and reduce colonisation of periodontopathogens to implant surfaces [131–133]. Immobilised histatin alone or immobilised conjugated peptides of histatin 5/titanium-binding peptide and lactoferricin/titanium-binding peptide reduce colonisation of *P. gingivalis* and enhance mRNA expression of Runx2, OPN and ALPase in osteoblastic cells.

4. Conclusions

Modified AMPs, including peptide mimetics, hybrid peptides, peptide congeners, stabilised peptides, peptide conjugates and immobilised peptides, have all emerged from natural AMPs. These unique classes of molecules are advertised to have selective or ‘targeted’ antimicrobial activities, improved retention, or unique abilities that allow them to bind to medical or industrial surfaces. These groups of new peptides have creative medical and industrial application potentials to treat antibiotic-resistant bacterial infections and septic shock, to preserve food, or to sanitise surfaces both in vitro and in vivo. It is possible that these molecules will become the next generation of peptides with potential as pharmaceuticals.

Acknowledgments

Funding

This review was supported by funds from the National Institutes of Health (NIH) National Institute of Dental and Craniofacial Research (NIDCR) (grant R01 DE014390).

References

1. Maróti G, Kereszt A, Kondorosi E, Mergaert P. Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol. 2011; 162:363–374. [PubMed: 21320593]
2. Hadley EB, Hancock RE. Strategies for the discovery and advancement of novel cationic antimicrobial peptides. Curr Top Med Chem. 2010; 10:1872–1881. [PubMed: 20615190]
3. Bowdish DM, Davidson DJ, Hancock RE. A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci. 2005; 6:35–51. [PubMed: 15638767]
4. Kohlgraf KG, Ackermann A, Lu X, Burnell K, Belanger M, Cavanaugh JE, et al. Defensins attenuate cytokine responses yet enhance antibody responses to *Porphyromonas gingivalis* adhesins in mice. Future Microbiol. 2010; 5:115–125. [PubMed: 20020833]
5. Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu Rev Immunol. 2004; 22:181–215. [PubMed: 15032578]
6. Diamond G, Ryan L. β-Defensins: what are they REALLY doing in the oral cavity? Oral Dis. 2011 Feb.18 [Epub ahead of print].
7. Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol. 2006; 6:468–472. [PubMed: 16890021]
8. Steinstraesser L, Kraneburg U, Jacobsen F, Al-Benna S. Host defense peptides and their antimicrobial–immunomodulatory duality. Immunobiology. 2011; 216:322–333. [PubMed: 20828865]
9. Hancock RE, Patrzykat A. Clinical development of cationic antimicrobial peptides: from natural to novel antibiotics. Curr Drug Targets Infect Disord. 2002; 2:79–83. [PubMed: 12462155]
10. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006; 24:1551–1557. [PubMed: 17160061]
11. Stevenson CL. Advances in peptide pharmaceuticals. Curr Pharm Biotechnol. 2009; 10:122–137. [PubMed: 19149594]
12. Tossi, A. Design and engineering strategies for synthetic antimicrobial peptides. In: Drider, D.; Rebuffat, S., editors. Prokaryotic antimicrobial peptides. From genes to applications. New York, NY: Springer; 2011. p. 81-98.
13. Brogden, KA. Perspectives and peptides of the next generation. In: Drider, D.; Rebuffat, S., editors. Prokaryotic antimicrobial peptides. From genes to applications. New York, NY: Springer; 2011. p. 423-439.
14. Findlay B, Zhanel GG, Schweizer F. Cationic amphiphiles, a new generation of antimicrobials inspired by the natural antimicrobial peptide scaffold. Antimicrob Agents Chemother. 2010; 54:4049–4058. [PubMed: 20696877]
15. Thaker HD, Sgolastra F, Clements D, Scott RW, Tew GN. Synthetic mimics of antimicrobial peptides from triaryl scaffolds. J Med Chem. 2011; 54:2241–2254. [PubMed: 21388190]
16. Palermo EF, Kuroda K. Structural determinants of antimicrobial activity in polymers which mimic host defense peptides. Appl Microbiol Biotechnol. 2010; 87:1605–1615. [PubMed: 20563718]
17. Olsen CA. Peptoid–peptide hybrid backbone architectures. Chembiochem. 2010; 11:152–160. [PubMed: 20017179]
18. Otvos L Jr, Snyder C, Condie BA, Bulet P, Wade JD. Chimeric antimicrobial peptides exhibit multiple modes of action. Int J Peptide Res Ther. 2005; 11:29–42.
19. Otvos L Jr. Antibacterial peptides and proteins with multiple cellular targets. J Pept Sci. 2005; 11:697–706. [PubMed: 16059966]
20. Takahashi D, Shukla SK, Prakash O, Zhang G. Structural determinants of host defense peptides for antimicrobial activity and target cell selectivity. Biochimie. 2010; 92:1236–1241. Erratum in: Biochimie 2011;93:631. [PubMed: 20188791]
21. Mookherjee N, Rehaume LM, Hancock RE. Cathelicidins and functional analogues as antisepsis molecules. Expert Opin Ther Targets. 2007; 11:993–1004. [PubMed: 17665972]

22. Garcia AE, Camarero JA. Biological activities of natural and engineered cyclotides, a novel molecular scaffold for peptide-based therapeutics. Curr Mol Pharmacol. 2010; 3:153–163. [PubMed: 20858197]
23. Jagadish K, Camarero JA. Cyclotides, a promising molecular scaffold for peptide-based therapeutics. Biopolymers. 2010; 94:611–616. [PubMed: 20564025]
24. Craik DJ, Conibear AC. The chemistry of cyclotides. J Org Chem. 2011 May.19 [Epub ahead of print].
25. Ireland DC, Clark RJ, Daly NL, Craik DJ. Isolation, sequencing, and structure–activity relationships of cyclotides. J Nat Prod. 2010; 73:1610–1622. [PubMed: 20718473]
26. Khare P, Jain A, Gulbake A, Soni V, Jain NK, Jain SK. Bioconjugates: harnessing potential for effective therapeutics. Crit Rev Ther Drug Carrier Syst. 2009; 26:119–155. [PubMed: 19673689]
27. Yacoby I, Benhar I. Targeted anti bacterial therapy. Infect Disord Drug Targets. 2007; 7:221–229. [PubMed: 17897058]
28. Costa F, Carvalho IF, Montelaro RC, Gomes P, Martins MC. Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces. Acta Biomater. 2011; 7:1431–1440. [PubMed: 21056701]
29. Onaizi SA, Leong SS. Tethering antimicrobial peptides: current status and potential challenges. Biotechnol Adv. 2011; 29:67–74. [PubMed: 20817088]
30. Takada H, Kotani S. Structural requirements of lipid A for endotoxicity and other biological activities. Crit Rev Microbiol. 1989; 16:477–523. [PubMed: 2663021]
31. Tossi A, Tarantino C, Romeo D. Design of synthetic antimicrobial peptides based on sequence analogy and amphipathicity. Eur J Biochem. 1997; 250:549–558. [PubMed: 9428709]
32. Chongsiriwatana NP, Miller TM, Wetzler M, Vakulenko S, Karlsson AJ, Palecek SP, et al. Short alkylated peptoid mimics of antimicrobial lipopeptides. Antimicrob Agents Chemother. 2011; 55:417–420. [PubMed: 20956607]
33. Kapoor R, Eimerman PR, Hardy JW, Cirillo JD, Contag CH, Barron AE. Efficacy of antimicrobial peptoids against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2011; 55:3058–3062. [PubMed: 21464254]
34. Kapoor R, Wadman MW, Dohm MT, Czyzewski AM, Spormann AM, Barron AE. Antimicrobial peptoids are effective against *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2011; 55:3054–3057. [PubMed: 21422218]
35. Radzishevsky IS, Rotem S, Bourdetsky D, Navon-Venezia S, Carmeli Y, Mor A. Improved antimicrobial peptides based on acyl-lysine oligomers. Nat Biotechnol. 2007; 25:657–659. [PubMed: 17529972]
36. Zaknoon F, Sarig H, Rotem S, Livne L, Ivankin A, Gidalevitz D, et al. Antibacterial properties and mode of action of a short acyl-lysyl oligomer. Antimicrob Agents Chemother. 2009; 53:3422–3429. [PubMed: 19487442]
37. Hua J, Yamarthy R, Felsenstein S, Scott RW, Markowitz K, Diamond G. Activity of antimicrobial peptide mimetics in the oral cavity: I. Activity against biofilms of *Candida albicans*. Mol Oral Microbiol. 2010; 25:418–425. [PubMed: 21040515]
38. Hua J, Scott RW, Diamond G. Activity of antimicrobial peptide mimetics in the oral cavity: II. Activity against periopathogenic biofilms and anti-inflammatory activity. Mol Oral Microbiol. 2010; 25:426–432. [PubMed: 21040516]
39. Held-Kuznetsov V, Rotem S, Assaraf YG, Mor A. Host-defense peptide mimicry for novel antitumor agents. FASEB J. 2009; 23:4299–4307. [PubMed: 19706728]
40. Rotem S, Mor A. Antimicrobial peptide mimics for improved therapeutic properties. Biochim Biophys Acta. 2009; 1788:1582–1592. [PubMed: 19028449]
41. Lienkamp K, Tew GN. Synthetic mimics of antimicrobial peptides—a versatile ring-opening metathesis polymerization based platform for the synthesis of selective antibacterial and cell-penetrating polymers. Chemistry. 2009; 15:11784–11800. [PubMed: 19798714]
42. Goldfeder Y, Zaknoon F, Mor A. Experimental conditions that enhance potency of an antibacterial oligo-acyl-lysyl. Antimicrob Agents Chemother. 2010; 54:2590–2595. [PubMed: 20385856]

43. Sarig H, Rotem S, Ziserman L, Danino D, Mor A. Impact of self-assembly properties on antibacterial activity of short acyl-lysine oligomers. Antimicrob Agents Chemother. 2008; 52:4308–4314. [PubMed: 18838600]
44. Piers KL, Brown MH, Hancock RE. Improvement of outer membrane-permeabilizing and lipopolysaccharide-binding activities of an antimicrobial cationic peptide by C-terminal modification. Antimicrob Agents Chemother. 1994; 38:2311–2316. [PubMed: 7840562]
45. Piers KL, Brown MH, Hancock REW. Recombinant DNA procedures for producing small antimicrobial cationic peptides in bacteria. Gene. 1993; 134:7–13. [PubMed: 8244033]
46. Piers KL, Hancock REW. The interaction of a recombinant cecropin/melittin hybrid peptide with the outer membrane of *Pseudomonas aeruginosa*. Mol Microbiol. 1994; 12:951–958. [PubMed: 7934902]
47. Wade D, Andreu D, Mitchell SA, Silveira AM, Boman A, Boman HG, et al. Antibacterial peptides designed as analogs or hybrids of cecropins and melittin. Int J Pept Protein Res. 1992; 40:429–436. [PubMed: 1483838]
48. Steiner H, Andreu D, Merrifield RB. Binding and action of cecropin and cecropin analogues: antibacterial peptides from insects. Biochim Biophys Acta. 1988; 939:260–266. [PubMed: 3128324]
49. Rozgonyi F, Szabo D, Kocsis B, Ostorhazi E, Abbadessa G, Cassone M, et al. The antibacterial effect of a proline-rich antibacterial peptide A3-APO. Curr Med Chem. 2009; 16:3996–4002. [PubMed: 19747127]
50. Szabo D, Ostorhazi E, Binas A, Rozgonyi F, Kocsis B, Cassone M, et al. The designer proline-rich antibacterial peptide A3-APO is effective against systemic *Escherichia coli* infections in different mouse models. Int J Antimicrob Agents. 2010; 35:357–361. [PubMed: 20031377]
51. Qiu XQ, Wang H, Lu XF, Zhang J, Li SF, Cheng G, et al. An engineered multidomain bactericidal peptide as a model for targeted antibiotics against specific bacteria. Nat Biotechnol. 2003; 21:1480–1485. [PubMed: 14625561]
52. Qiu XQ, Zhang J, Wang H, Wu GY. A novel engineered peptide, a narrow-spectrum antibiotic, is effective against vancomycin-resistant *Enterococcus faecalis*. Antimicrob Agents Chemother. 2005; 49:1184–1189. [PubMed: 15728923]
53. Eckert R, He J, Yarbrough DK, Qi F, Anderson MH, Shi W. Targeted killing of *Streptococcus mutans* by a pheromone-guided 'smart' antimicrobial peptide. Antimicrob Agents Chemother. 2006; 50:3651–3657. [PubMed: 17060534]
54. He J, Anderson MH, Shi W, Eckert R. Design and activity of a 'dual-targeted' antimicrobial peptide. Int J Antimicrob Agents. Int 2009; 33:532–537. [PubMed: 19188046]
55. Saugar JM, Alarcon T, Lopez-Hernandez S, Lopez-Brea M, Andreu D, Rivas L. Activities of polymyxin B and cecropin A–melittin peptide $CA_{1–8}M_{1–18}$ against a multiresistant strain of *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2002; 46:875–878. [PubMed: 11850277]
56. Yevtushenko DP, Romero R, Forward BS, Hancock RE, Kay WW, Misra S. Pathogen-induced expression of a cecropin A–melittin antimicrobial peptide gene confers antifungal resistance in transgenic tobacco. J Exp Bot. 2005; 56:1685–1695. [PubMed: 15863447]
57. Osusky M, Zhou G, Osuska L, Hancock RE, Kay WW, Misra S. Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. Nat Biotechnol. 2000; 18:1162–1166. [PubMed: 11062434]
58. Bratt CL, Kohlgraf KG, Yohnke K, Kummet C, Dawson DV, Brogden KA. Communication: Antimicrobial activity of SMAP28 with a targeting domain for *Porphyromonas gingivalis*. Probiotics Antimicrob Proteins. 2009; 2:21–25. [PubMed: 20454638]
59. Larrick JW, Hirata M, Shimomoura Y, Yoshida M, Zheng H, Zhong J, et al. Antimicrobial activity of rabbit CAP18-derived peptides. Antimicrob Agents Chemother. 1993; 37:2534–2539. [PubMed: 8109914]
60. Larrick JW, Hirata M, Zheng H, Zhong J, Bolin D, Cavaillon JM, et al. A novel granulocyte-derived peptide with lipopolysaccharide-neutralizing activity. J Immunol. 1994; 152:231–240. [PubMed: 8254193]

61. Larrick JW, Hirata M, Zhong J, Wright SC. Anti-microbial activity of human CAP18 peptides. Immunotechnology. 1995; 1:65–72. [PubMed: 9373334]
62. Hirata M, Shimomura Y, Yoshida M, Morgan JG, Palings I, Wilson D, et al. Characterization of a rabbit cationic protein (CAP18) with lipopolysaccharide-inhibitory activity. Infect Immun. 1994; 62:1421–1426. [PubMed: 8132348]
63. Tasaka S, Ishizaka A, Urano T, Sayama K, Sakamaki F, Nakamura H, et al. A derivative of cationic antimicrobial protein attenuates lung injury by suppressing cell adhesion. Am J Respir Cell Mol Biol. 1996; 15:738–744. [PubMed: 8969268]
64. Sawa T, Kurahashi K, Ohara M, Gropper MA, Doshi V, Larrick JW, et al. Evaluation of antimicrobial and lipopolysaccharide-neutralizing effects of a synthetic CAP18 fragment against *Pseudomonas aeruginosa* in a mouse model. Antimicrob Agents Chemother. 1998; 42:3269–3275. [PubMed: 9835525]
65. Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, et al. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect Immun. 2000; 68:2748–2755. [PubMed: 10768969]
66. Larrick JW, Lee J, Ma S, Li X, Francke U, Wright SC, et al. Structural, functional analysis and localization of the human CAP18 gene. FEBS Lett. 1996; 398:74–80. [PubMed: 8946956]
67. Ciornei CD, Sigurdardottir T, Schmidtchen A, Bodelsson M. Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37. Antimicrob Agents Chemother. 2005; 49:2845–2850. [PubMed: 15980359]
68. Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. Infect Immun. 1995; 63:1291–1297. [PubMed: 7890387]
69. Bagella L, Scocchi M, Zanetti M. cDNA sequences of three sheep myeloid cathelicidins. FEBS Lett. 1995; 376:225–228. [PubMed: 7498547]
70. Brogden KA, Kalfa VC, Ackermann MR, Palmquist DE, McCray PB Jr, Tack BF. The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection. Antimicrob Agents Chemother. 2001; 45:331–334. [PubMed: 11120991]
71. Kalfa VC, Jia HP, Kunkle RA, McCray PB Jr, Tack BF, Brogden KA. Congeners of SMAP29 kill ovine pathogens and induce ultrastructural damage in bacterial cells. Antimicrob Agents Chemother. 2001; 45:3256–3261. [PubMed: 11600395]
72. Mahoney MM, Lee AY, Brezinski-Caliguri DJ, Huttner KM. Molecular analysis of the sheep cathelin family reveals a novel antimicrobial peptide. FEBS Lett. 1995; 377:519–522. [PubMed: 8549789]
73. Saiman L, Tabibi S, Starner TD, San Gabriel P, Winokur PL, Jia HP, et al. Cathelicidin peptides inhibit multiply antibiotic-resistant pathogens from patients with cystic fibrosis. Antimicrob Agents Chemother. 2001; 45:2838–2844. [PubMed: 11557478]
74. Skerlavaj B, Benincasa M, Risso A, Zanetti M, Gennaro R. SMAP-29: a potent antibacterial and antifungal peptide from sheep leukocytes. FEBS Lett. 1999; 463:58–62. [PubMed: 10601638]
75. Weistroffer PL, Joly S, Srikantha R, Tack BF, Brogden KA, Guthmiller JM. SMAP29 congeners demonstrate activity against oral bacteria and reduced toxicity against oral keratinocytes. Oral Microbiol Immunol. 2008; 23:89–95. [PubMed: 18279175]
76. Sawai MV, Waring AJ, Kearney WR, McCray PB Jr, Forsyth WR, Lehrer RI, et al. Impact of single-residue mutations on the structure and function of ovispirin/novispirin antimicrobial peptides. Protein Eng. 2002; 15:225–232. [PubMed: 11932493]
77. Dawson RM, Liu CQ. Analogues of peptide SMAP-29 with comparable antimicrobial potency and reduced cytotoxicity. Int J Antimicrob Agents. 2011; 37:432–437. [PubMed: 21377841]
78. Saravanan R, Bhunia A, Bhattacharjya S. Micelle-bound structures and dynamics of the hinge deleted analog of melittin and its diastereomer: implications in cell selective lysis by d-amino acid containing antimicrobial peptides. Biochim Biophys Acta. 2010; 1798:128–139. [PubMed: 19635451]
79. Xiong YQ, Bayer AS, Elazegui L, Yeaman MR. A synthetic congener modeled on a microbicidal domain of thrombin-induced platelet microbicidal protein 1 recapitulates staphylocidal

mechanisms of the native molecule. Antimicrob Agents Chemother. 2006; 50:3786–3792. [PubMed: 16954324]

80. Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. Nat Immunol. 2005; 6:551–557. [PubMed: 15908936]
81. Lehrer RI. Primate defensins. Nat Rev Microbiol. 2004; 2:727–738. [PubMed: 15372083]
82. Schroeder BO, Wu Z, Nuding S, Groscurth S, Marcinowski M, Beisner J, et al. Reduction of disulphide bonds unmasks potent antimicrobial activity of human β-defensin 1. Nature. 2011; 469:419–423. [PubMed: 21248850]
83. De Cecco M, Seo ES, Clarke DJ, McCullough BJ, Taylor K, Macmillan D, et al. Conformational preferences of linear β-defensins are revealed by ion mobility–mass spectrometry. J Phys Chem B. 2010; 114:2312–2318. [PubMed: 20102218]
84. Hoover DM, Wu Z, Tucker K, Lu W, Lubkowski J. Antimicrobial characterization of human β-defensin 3 derivatives. Antimicrob Agents Chemother. 2003; 47:2804–2809. [PubMed: 12936977]
85. Wu Z, Hoover DM, Yang D, Boulegue C, Santamaria F, Oppenheim JJ, et al. Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human β-defensin 3. Proc Natl Acad Sci U S A. 2003; 100:8880–8885. [PubMed: 12840147]
86. Liu S, Zhou L, Li J, Suresh A, Verma C, Foo YH, et al. Linear analogues of human β-defensin 3: concepts for design of antimicrobial peptides with reduced cytotoxicity to mammalian cells. Chembiochem. 2008; 9:964–973. [PubMed: 18350527]
87. Taylor K, Clarke DJ, McCullough B, Chin W, Seo E, Yang D, et al. Analysis and separation of residues important for the chemoattractant and antimicrobial activities of β-defensin 3. J Biol Chem. 2008; 283:6631–6639. [PubMed: 18180295]
88. Chandrababu KB, Ho B, Yang D. Structure, dynamics, and activity of an all-cysteine mutated human β defensin-3 peptide analogue. Biochemistry. 2009; 48:6052–6061. [PubMed: 19480463]
89. Jung S, Mysliwy J, Spudy B, Lorenzen I, Reiss K, Gelhaus C, et al. Human β-defensin 2 and β-defensin 3 chimeric peptides reveal the structural basis of the pathogen specificity of their parent molecules. Antimicrob Agents Chemother. 2011; 55:954–960. [PubMed: 21189349]
90. Scudiero O, Galdiero S, Cantisani M, Di Noto R, Vitiello M, Galdiero M, et al. Novel synthetic, salt-resistant analogs of human β-defensins 1 and 3 endowed with enhanced antimicrobial activity. Antimicrob Agents Chemother. 2010; 54:2312–2322. [PubMed: 20308372]
91. Reynolds NL, De Cecco M, Taylor K, Stanton C, Kilanowski F, Kalapothakis J, et al. Peptide fragments of a β-defensin derivative with potent bactericidal activity. Antimicrob Agents Chemother. 2010; 54:1922–1929. [PubMed: 20176896]
92. Bai Y, Liu S, Jiang P, Zhou L, Li J, Tang C, et al. Structure-dependent charge density as a determinant of antimicrobial activity of peptide analogues of defensin. Biochemistry. 2009; 48:7229–7239. [PubMed: 19580334]
93. Krishnakumari V, Rangaraj N, Nagaraj R. Antifungal activities of human β-defensins HBD-1 to HBD-3 and their C-terminal analogs Phd1 to Phd3. Antimicrob Agents Chemother. 2009; 53:256–260. [PubMed: 18809937]
94. Salvucci E, Saavedra L, Sesma F. Short peptides derived from the NH2-terminus of subclass IIa bacteriocin enterocin CRL35 show antimicrobial activity. J Antimicrob Chemother. 2007; 59:1102–1108. [PubMed: 17449885]
95. Suzuki MM, Matsumoto M, Yamamoto A, Ochiai M, Horiuchi Y, Niwa M, et al. Molecular design of LPS-binding peptides. J Microbiol Methods. 2010; 83:153–155. [PubMed: 20816904]
96. Futaki S, Suzuki T, Ohashi W, Yagami T, Tanaka S, Ueda K, et al. Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J Biol Chem. 2001; 276:5836–5840. [PubMed: 11084031]
97. Kichler A, Leborgne C, Marz J, Danos O, Bechinger B. Histidine-rich amphipathic peptide antibiotics promote efficient delivery of DNA into mammalian cells. Proc Natl Acad Sci U S A. 2003; 100:1564–1568. [PubMed: 12563034]
98. Kim YC, Ludovice PJ, Prausnitz MR. Transdermal delivery enhanced by antimicrobial peptides. J Biomed Nanotechnol. 2010; 6:612–620. [PubMed: 21329054]

99. Pasupuleti M, Chalupka A, Morgelin M, Schmidtchen A, Malmsten M. Tryptophan end-tagging of antimicrobial peptides for increased potency against *Pseudomonas aeruginosa*. Biochim Biophys Acta. 2009; 1790:800–808. [PubMed: 19345721]
100. Catiau L, Traisnel J, Delval-Dubois V, Chihib NE, Guillochon D, Nedjar-Arroume N. Minimal antimicrobial peptidic sequence from hemoglobin α-chain: KYR. Peptides. 2011; 32:633–638. [PubMed: 21262306]
101. Mathison RD, Davison JS, Befus AD, Gingerich DA. Salivary gland derived peptides as a new class of anti-inflammatory agents: review of preclinical pharmacology of C-terminal peptides of SMR1 protein. J Inflamm (Lond). 2010; 7:49. [PubMed: 20920210]
102. Tam JP, Lu YA, Yang JL, Chiu KW. An unusual structural motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides. Proc Natl Acad Sci U S A. 1999; 96:8913–8918. [PubMed: 10430870]
103. Yu Q, Lehrer RI, Tam JP. Engineered salt-insensitive -defensins with end-toend circularized structures. J Biol Chem. 2000; 275:3943–3949. [PubMed: 10660548]
104. Levengood MR, van der Donk WA. Use of lantibiotic synthetases for the preparation of bioactive constrained peptides. Bioorg Med Chem Lett. 2008; 18:3025–3028. [PubMed: 18294843]
105. Kluskens LD, Nelemans SA, Rink R, de Vries L, Meter-Arkema A, Wang Y, et al. Angiotensin-(1–7) with thioether bridge: an angiotensin-converting enzyme-resistant, potent angiotensin-(1–7) analog. J Pharmacol Exp Ther. 2009; 328:849–854. [PubMed: 19038778]
106. Abraham T, Marwaha S, Kobewka DM, Lewis RN, Prenner EJ, Hodges RS, et al. The relationship between the binding to and permeabilization of phospholipid bilayer membranes by GS14dK4, a designed analog of the antimicrobial peptide gramicidin S. Biochim Biophys Acta. 2007; 1768:2089–2098. [PubMed: 17686454]
107. Peschen D, Li HP, Fischer R, Kreuzaler F, Liao YC. Fusion proteins comprising a *Fusarium*-specific antibody linked to antifungal peptides protect plants against a fungal pathogen. Nat Biotechnol. 2004; 22:732–738. [PubMed: 15146196]
108. Franzman MR, Burnell KK, Dehkordi-Vakil FH, Guthmiller JM, Dawson DV, Brogden KA. Targeted antimicrobial activity of a specific IgG–SMAP28 conjugate against *Porphyromonas gingivalis* in a mixed culture. Int J Antimicrob Agents. 2009; 33:14–20. [PubMed: 18778918]
109. Leupold E, Nikolenko H, Dathe M. Apolipoprotein E peptide-modified colloidal carriers: the design determines the mechanism of uptake in vascular endothelial cells. Biochim Biophys Acta. 2009; 1788:442–449. [PubMed: 19121285]
110. An LL, Yang YH, Ma XT, Lin YM, Li G, Song YH, et al. LL-37 enhances adaptive antitumor immune response in a murine model when genetically fused with M-CSFR (J6-1) DNA vaccine. Leuk Res. 2005; 29:535–543. [PubMed: 15755506]
111. Biragyn A, Surenhu M, Yang D, Ruffini PA, Haines BA, Klyushnenkova E, et al. Mediators of innate immunity that target immature, but not mature, dendritic cells induce antitumor immunity when genetically fused with nonimmunogenic tumor antigens. J Immunol. 2001; 167:6644–6653. [PubMed: 11714836]
112. Bucki R, Leszczynska K, Byfield FJ, Fein DE, Won E, Cruz K, et al. Combined antibacterial and anti-inflammatory activity of a cationic disubstituted dexamethasone–spermine conjugate. Antimicrob Agents Chemother. 2010; 54:2525–2533. [PubMed: 20308375]
113. Chu-Kung AF, Nguyen R, Bozzelli KN, Tirrell M. Chain length dependence of antimicrobial peptide–fatty acid conjugate activity. J Colloid Interface Sci. 2010; 345:160–167.
114. Ding B, Taotofa U, Orsak T, Chadwell M, Savage PB. Synthesis and characterization of peptide–cationic steroid antibiotic conjugates. Org Lett. 2004; 6:3433–3436. [PubMed: 15387516]
115. Guyomard A, Dé E, Jouenne T, Malandain JJ, Muller GM, Glinel K. Incorporation of a hydrophobic antibacterial peptide into amphiphilic polyelectrolyte multilayers: a bioinspired approach to prepare biocidal thin coatings. Adv Funct Mater. 2008; 18:758–765.
116. Cutter CN, Willett JL, Siragusa GR. Improved antimicrobial activity of nisin-incorporated polymer films by formulation change and addition of food grade chelator. Lett Appl Microbiol. 2001; 33:325–328. [PubMed: 11559410]
117. Etienne O, Picart C, Taddei C, Haikel Y, Dimarcq JL, Schaaf P, et al. Multilayer polyelectrolyte films functionalized by insertion of defensin: a new approach to protection of implants from

bacterial colonization. Antimicrob Agents Chemother. 2004; 48:3662–3669. [PubMed: 15388417]

118. Bagheri M, Beyermann M, Dathe M. Immobilization reduces the activity of surface-bound cationic antimicrobial peptides with no influence upon the activity spectrum. Antimicrob Agents Chemother. 2009; 53:1132–1141. [PubMed: 19104020]
119. Haynie SL, Crum GA, Doele BA. Antimicrobial activities of amphiphilic peptides covalently bonded to a water-insoluble resin. Antimicrob Agents Chemother. 1995; 39:301–307. [PubMed: 7726486]
120. Glinel K, Jonas AM, Jouenne T, Leprince J, Galas L, Huck WT. Antibacterial and antifouling polymer brushes incorporating antimicrobial peptide. Bioconjug Chem. 2009; 20:71–77. [PubMed: 19113948]
121. Gao G, Lange D, Hilpert K, Kindrachuk J, Zou Y, Cheng JT, et al. The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. Biomaterials. 2011; 32:3899–3909. [PubMed: 21377727]
122. Rotem S, Raz N, Kashi Y, Mor A. Bacterial capture by peptide-mimetic oligoacyllysine surfaces. Appl Environ Microbiol. 2011; 76:3301–3307. [PubMed: 20363797]
123. Guerra NP, Araujo AB, Barrera AM, Agrasar AT, Macias CL, Carballo J, et al. Antimicrobial activity of nisin adsorbed to surfaces commonly used in the food industry. J Food Prot. 2005; 68:1012–1019. [PubMed: 15895735]
124. Hequet A, Humblot V, Berjeaud JM, Pradier CM. Optimized grafting of antimicrobial peptides on stainless steel surface and biofilm resistance tests. Colloids Surf B Biointerfaces. 2011; 84:301–309. [PubMed: 21310597]
125. Luckarift HR, Dickerson MB, Sandhage KH, Spain JC. Rapid, room-temperature synthesis of antibacterial bionanocomposites of lysozyme with amorphous silica or titania. Small. 2006; 2:640–643. [PubMed: 17193101]
126. Izquierdo-Barba I, Vallet-Regi M, Kupferschmidt N, Terasaki O, Schmidtchen A, Malmsten M. Incorporation of antimicrobial compounds in mesoporous silica film monolith. Biomaterials. 2009; 30:5729–5736. [PubMed: 19628277]
127. Kulagina NV, Lassman ME, Ligler FS, Taitt CR. Antimicrobial peptides for detection of bacteria in biosensor assays. Anal Chem. 2005; 77:6504–6508. [PubMed: 16194120]
128. Kulagina NV, Shaffer KM, Anderson GP, Ligler FS, Taitt CR. Antimicrobial peptide-based array for *Escherichia coli* and *Salmonella* screening. Anal Chim Acta. 2006; 575:9–15. [PubMed: 17723565]
129. Taitt CR, North SH, Kulagina NV. Antimicrobial peptide arrays for detection of inactivated biothreat agents. Methods Mol Biol. 2009; 570:233–255. [PubMed: 19649597]
130. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol. 2005; 3:238–250. [PubMed: 15703760]
131. Yoshinari M, Kato T, Matsuzaka K, Hayakawa T, Inoue T, Oda Y, et al. Adsorption behavior of antimicrobial peptide histatin 5 on PMMA. J Biomed Mater Res B Appl Biomater. 2006; 77:47–54. [PubMed: 16206258]
132. Makihira S, Shuto T, Nikawa H, Okamoto K, Mine Y, Takamoto Y, et al. Titanium immobilized with an antimicrobial peptide derived from histatin accelerates the differentiation of osteoblastic cell line, MC3T3-E1. Int J Mol Sci. 2010; 11:1458–1470. [PubMed: 20480030]
133. Yoshinari M, Kato T, Matsuzaka K, Hayakawa T, Shiba K. Prevention of biofilm formation on titanium surfaces modified with conjugated molecules comprised of antimicrobial and titanium-binding peptides. Biofouling. 2010; 26:103–110. [PubMed: 20390560]

Table 1

Relevant and recent reviews of composition, structure and activities of modified antimicrobial peptides

	Reference(s)
1. Introduction	
Advances in peptide pharmaceuticals	[11]
2. Rational design of modified antimicrobial peptides	
Design and engineering strategies for synthetic antimicrobial peptides	[12,13]
3. Modified antimicrobial peptides	
3.1. Antimicrobial peptide mimetics	
Mimetics as a new generation of antimicrobials inspired by the natural antimicrobial peptide and triaryl scaffolds	[14,15]
Structural determinants of antimicrobial activity in polymers	[16]
Peptoid–peptide hybrid backbone architectures	[17]
3.2. Hybrid antimicrobial peptides	
Chimeric antimicrobial peptides exhibit multiple modes of action	[18,19]
3.3. Antimicrobial peptide congeners	
Structural determinants of host defence peptides for antimicrobial activity and target cell selectivity	[20]
Cathelicidins and functional analogues as antisepsis molecules	[21]
3.4. Cyclotides and stabilised antimicrobial peptides	
Biological activities of natural and engineered cyclotides, a novel molecular scaffold for peptide-based therapeutics	[22]
The chemistry of cyclotides	[22–25]
3.5. Antimicrobial peptide conjugates	
Polymer-based, macromolecule-based, carrier-based and novel bioconjugates	[26]
‘Targeted’ antibacterial therapy	[27]
3.6. Immobilised antimicrobial peptides	
Covalent immobilisation of antimicrobial peptides onto biomaterial surfaces	[28]
Tethering antimicrobial peptides: current status and potential challenges	[29]

Table 2

Diverse biological activities of peptide mimetics

Activity	Example	Reference(s)
Antimicrobial activity		
Peptoids	Peptoid 1-C13$_{4mer}$ is active against *Mycobacterium bovis* bacille Calmette–Guérin and *Mycobacterium tuberculosis*, and peptoid 1 and 1-C13$_{4mer}$ are active against *Pseudomonas aeruginosa* biofilms. Ndec-1$_{6mer}$ and Ntridec-1$_{4mer}$ are active against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*	[16,32–34]
Oligo-acyl-lysine (OAK) derivatives	C_{12}K-7α_8 is active in vitro and in vivo against Gram-negative bacteria, with no haemolytic activity	[35]
	NC_{12}-2 β_{12} exerts potent activity against Gram-positive bacteria whilst exhibiting negligible haemolytic activity	[36]
Phenylalkyne and arylamide compounds	Potent antifungal activity against *Candida* spp. both in planktonic and biofilm forms as well as in the presence of saliva	[37]
Phenylalkyne and arylamide compounds	mPE exhibited potent and rapid antimicrobial activity against biofilms of both *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*	[38]
Antitumour activity		
Oligo-acyl-lysine (OAK) derivatives	α_{12}-3β_{12} exhibits dose-dependent inhibition of TRAMP-C2, LNCAP, PC3, MCF-7 and N-417 tumour cell lines in vitro and tumour growth in vivo in mice	[39]
Anti-inflammatory activity		
Phenylalkyne and arylamide compounds	Cell cultures treated with mPE demonstrate dose-dependent inhibition of interleukin (IL)-8 secretion, suggesting mPE is an anti-inflammatory agent, possibly by interfering with NF-κB signal transduction	[38]

Table 3

Diverse biological activities of hybrid peptides

Activity	Example	Reference(s)
Increased antimicrobial activity	CEME, CEMA, CP26 and CP29 permeabilise the outer membrane of *Pseudomonas aeruginosa*, bind lipopolysaccharide and bind lipoteichoic acid	[44–48]
	A3-APO has a 'dual mode of action', attacking both the bacterial membrane and intracellular heat shock protein DnaK	[49,50]
Targeted antimicrobial activity	Microbial pheromones (e.g. staphylococcal AgrD1, enterococcal cCF109, streptococcal competence stimulating peptide) mediate microorganism-specific delivery of an antimicrobial peptide domain (e.g. channel-forming domain of colicin Ia)	[51–54]
	$M8_{KH}$-20 displays specific targeted activity against *P. aeruginosa* and *Streptococcus mutans*	[54]
Antitumour activity	Cecropin A(1–8) and magainin Z(1–12) have high antitumor activity and less haemolytic activity	[55]

Table 4

Diverse biological activities of congener peptides

Peptide	Example	Reference(s)
CAP18	Congeners of $CAP18_{106–142}$ have antimicrobial activity, inhibit the binding of LPS, inhibit LPS activation of mouse macrophages and human monocytes, inhibit LPS-induced release of cytokines and nitric oxide from macrophages, inhibit LPS-induced *Limulus* amoebocyte lysate coagulation, and protect mice from LPS lethality	[59–65]
LL-37 ($hCAP18_{104–140}$)	Congeners of LL-37 have antimicrobial activity (even in 175 mM NaCl); inhibit the binding of LPS, inhibit LPS-induced release of nitric oxide from macrophages and inhibit LPS-induced vascular nitric oxide production; attract neutrophil granulocytes; retain activity in serum; induce less haemolysis; and protect mice from LPS lethality	[61,65–68]
SMAP29	SMAP28, ovispirin, novispirin and other congeners have varying degrees of antimicrobial activity, are active both in low and high ionic strength conditions, induce significant morphological alterations in bacterial surfaces, and reduce the concentration of bacteria in models of infection	[65,69–77]
Melittin	Deletion of the hinge amino acid residues along with two C-terminal terminal glutamine residues (Q25 and Q26) yields a peptide analogue of 19 amino acid residues, does not reduce antibacterial activity but does reduce haemolytic activity	[78]
Thrombin-induced platelet microbicidal protein 1	A synthetic congener, called RP-1, has rapid bactericidal activity, induces extensive membrane permeabilisation without depolarisation, and inhibits DNA, RNA and protein synthesis	[79]

LPS, lipopolysaccharide.

Table 5

Diverse biological activities of peptide conjugates

Activity	Example	Reference(s)
Antimicrobial activity	Antifungal peptides linked to a *Fusarium* spp.-specific antibody	[107]
	SMAP28 linked to rabbit IgG antibodies, specific to the outer surface of *Porphyromonas gingivalis* strain 381	[108]
	Apolipoprotein E-derived peptide efficiently translocates across cell membranes	[109]
Antitumour activity	LL-37 enhances an antitumor immune response of M-CSFR$_{J6\text{-}1}$, a potential target for tumour immunotherapy, when LL-37 is genetically fused with M-CSFR$_{J6\text{-}1}$ and administered to mice	[110]
	Mouse β-defensin linked to a tumour antigen elicits potent cell-mediated responses and antitumour immunity when genetically fused with another non-immunogenic tumour antigen	[111]

Table 6

Diverse biological activities of immobilised antimicrobial peptides

Antimicrobial peptide and activity	Reference(s)
Nisin, *Anopheles gambiae* mosquito defensin and gramicidin A retard spoilage and increase food preservation times when incorporated into plastics or films	[115–117]
Peptide KLAL, magainin-derived peptide MK5E, magainin I and Tet peptides (e.g. Tet-20, Tet-26, Tet-213 and 1010) can be tethered to resins or brush layers to make contact-active cationic antimicrobial surfaces. In some instances, immobilisation reduces antimicrobial activity but not the spectrum of activity	[118–121]
Oligo-acyl-lysine (OAK) and polylysines tethered to resins efficiently captures pathogens in different media in batch or flow-through procedures	[122]
Nisin, nisaplin (a congener of nisin) and magainin I can be directly adsorbed to surfaces to make contact-active cationic antimicrobial surfaces	[123,124]
LL-37 and lysozyme can be encapsulated within silica or titania nanoparticles to create bionanocomposite antifouling materials with broad-spectrum antimicrobial activities or for use as cosmetics with antimicrobial activities and sunscreen properties	[125,126]
Cecropin A, magainin I and parasin immobilised to glass can capture, concentrate and detect microorganisms in screening assays for the detection of food-borne pathogens or microbial biothreat agents	[127–129]