Journal of Microbiology, Immunology and Infection (2017) 50, 405–410

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Review Article

Antimicrobial peptides as potential anti-biofilm agents against multidrug-resistant bacteria

Pooi Yin Chung [a,*], **Ramona Khanum** [b]

[a] *Department of Pathology, School of Medicine, International Medical University, Kuala Lumpur, Malaysia*
[b] *Postgraduate Studies and Research, International Medical University, Kuala Lumpur, Malaysia*

Received 2 September 2016; received in revised form 20 October 2016; accepted 3 December 2016
Available online 26 June 2017

KEYWORDS
Antimicrobial peptide;
Biofilms;
Multidrug-resistant bacteria

Abstract Bacterial resistance to commonly used drugs has become a global health problem, causing increased infection cases and mortality rate. One of the main virulence determinants in many bacterial infections is biofilm formation, which significantly increases bacterial resistance to antibiotics and innate host defence. In the search to address the chronic infections caused by biofilms, antimicrobial peptides (AMP) have been considered as potential alternative agents to conventional antibiotics. Although AMPs are commonly considered as the primitive mechanism of immunity and has been extensively studied in insects and non-vertebrate organisms, there is now increasing evidence that AMPs also play a crucial role in human immunity. AMPs have exhibited broad-spectrum activity against many strains of Gram-positive and Gram-negative bacteria, including drug-resistant strains, and fungi. In addition, AMPs also showed synergy with classical antibiotics, neutralize toxins and are active in animal models. In this review, the important mechanisms of action and potential of AMPs in the eradication of biofilm formation in multidrug-resistant pathogen, with the goal of designing novel antimicrobial therapeutics, are discussed.

Introduction

Chronic wound infections as a result of pressure sores, venous legs ulcers and diabetic foot ulcers are typically caused by multiple genera of bacteria, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are strong biofilm formers.[1] The presence of biofilm has now been identified as the cause of poor healing of these

* Corresponding author. Department of Pathology, School of Medicine, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
E-mail address: katrina_chung@imu.edu.my (P.Y. Chung).

http://dx.doi.org/10.1016/j.jmii.2016.12.005

wounds. While physical debridement can assist the healing of these wounds, biofilm-focused therapeutic approaches can promote more rapid healing in a large percent of patients.[2] Thus, a biofilm-centric approach to reduce the ability of these pathogens to form biofilms is urgently needed to enable more effective subsequent healing by the body or treatment with antibiotics. In the search for an effective agent that can treat chronic infections, antimicrobial peptides (AMPs) have been shown to demonstrate antimicrobial, anti-attachment and anti-biofilm properties.

AMPs are essential components of innate immunity in humans and other higher organisms, contributing to the first line of defense against infections.[3] Despite co-evolution with bacteria, AMPs have retained their advantage and bacteria have yet to develop wide-spread resistance. As such, there is growing interest in the therapeutic application of these molecules. Their amino-acid sequences, net-positive charge, amphipathicity, and very small size allow AMPs to bind to and disrupt membranes of microbes. Other researches have shown that AMPs can also inhibit cell wall, nucleic acid, and protein biosynthesis.[4]

Classification of AMP

AMPs are generally made up of 10—50 amino-acid residues and are divided based on the composition of their amino-acid, size and conformational structures. Due to the increasing number of AMPs, there are 13 databases of AMPs to date, which manage information and conduct peptide analysis (Fig. 1).[5]

Structurally, AMPs can be classified in four major classes: β-sheet, α-helical, loop and extended peptides,[6] with the first two classes being the most common in nature. Among the best studied AMPs are magainins, magainin 2 and PGLa, which are α-helical peptides that were originally isolated from the skin of the African frog *Xenopus laevis*.[7] In humans, the two most well characterized families of host defence peptides are cathelicidins and defensins. Cathelicidins are AMPs bearing an amino-terminal cathepsin L inhibitor domain (cathelin), and produced by leukocytes,[8] while defensins are a highly complex group of open-ended cysteine-rich peptides widely distributed in nature and found both in vertebrates and invertebrates. On the basis of their size and pattern of disulfide bonding, mammalian defensins are classified into α-, β- and θ-defensins.[9]

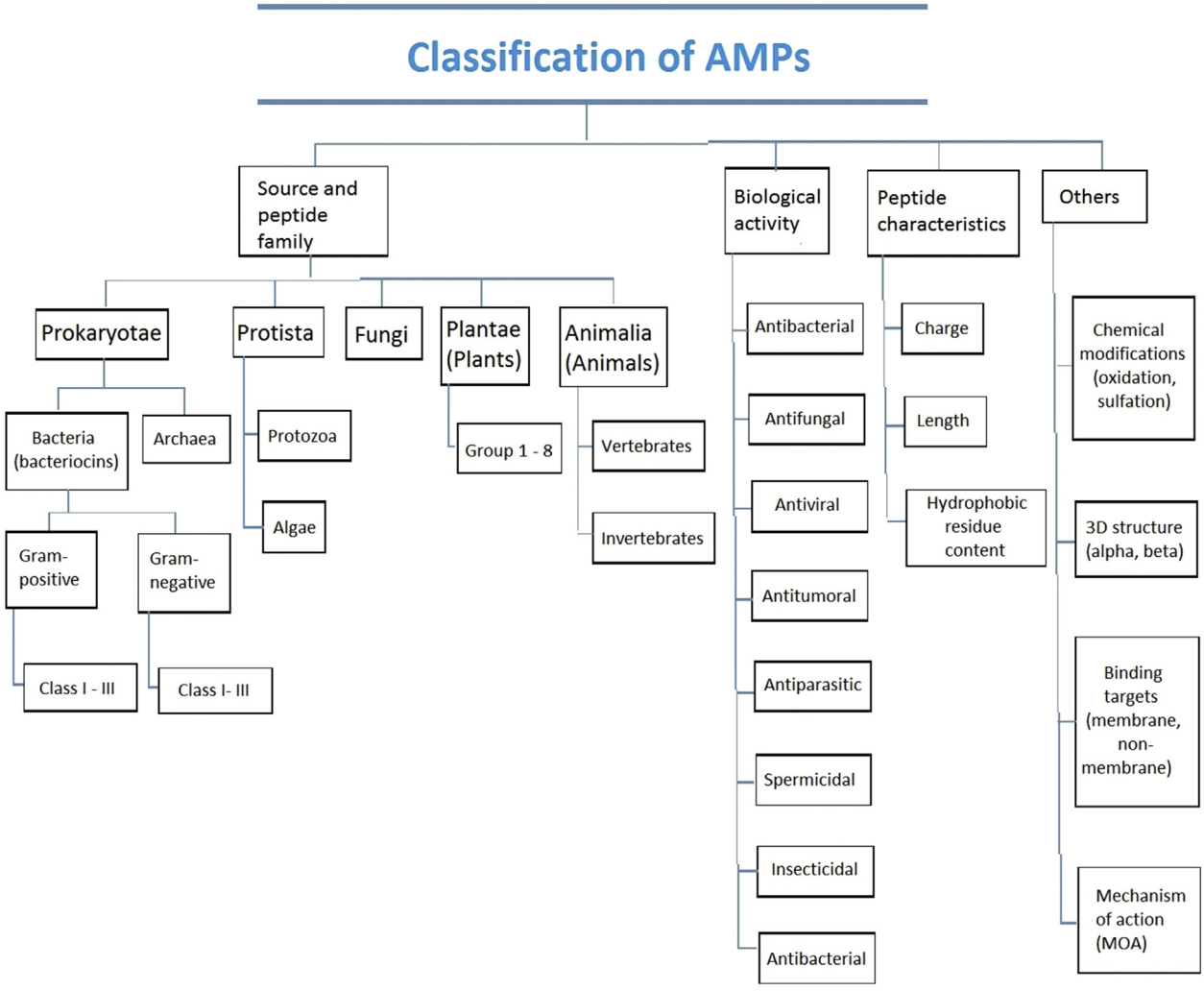

Figure 1. Classification of AMPs based on various referring factors.

Antimicrobial and anti-biofilm activities of AMP

AMPs are effector molecules of the innate immune system and have a broad antimicrobial spectrum. In addition to direct antimicrobial function, AMPs mediate the inflammatory response with impact on epithelial and inflammatory cells, resulting in cytokine release, cell proliferation, angiogenesis, wound healing and chemotaxis.[10] AMPs have consistently exhibited potent synergistic activity with clinically used antibiotics such as vancomycin, penicillin, ampicillin, β-lactams, polymyxin E, doxycycline, daptomycin, linezolid, teicoplanin, azithromycin, ciprofloxacin and clarithromycin.[11]

SMAP-29, a cathelicidin-derived peptide from sheep myeloid mRNA, exert potent antimicrobial activity against methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VREF) and mucoid *P. aeruginosa*.[12] A broad-spectrum antibacterial peptides isolated from *Enterococcus mundtii* ST4V have been shown to be active against multidrug-resistant Gram-positive and Gram-negative bacterium, including *Streptococcus* species, *P. aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *S. aureus*.[13]

Synthetic antimicrobial peptide (AMP) NA-CATH:ATRA1-ATRA1 and natural AMP LL-37 from the cathelicidin family inhibits the production of biofilms by *S. aureus* at concentrations lower than 3 μg/mL.[14] Lactoferrin, conjugated lactoferricin, melimine and citropin 1.1. have shown good anti-biofilm activity in medical devices infection against *S. aureus* and *P. aeruginosa*, particularly when administered together with conventional antibiotics, such as rifampicin and minocycline.[15] Lactoferrin's anti-biofilm activity is associated with the proteins' iron-chelating properties; increasing surface motility, i.e. twitching which is a specialized form of surface locomotion mediated by type 4 pili; and promoting the formation of thin, flat biofilms by surface-wandering bacterial cells, allowing these cells to be more susceptible to removal.[16] The human cationic host defence peptide, LL-37 decreases the attachment of *P. aeruginosa* cells onto the surface of medical devices and tissues, stimulates twitching motility mediated by type IV pili and down-regulates the Las and Rhl QS system.[17] LL-37 also inhibits initial attachment and development of biofilm in *Staphylococcus epidermidis*.[18] A recent study with four chimeric AMPs against biofilms of multidrug-resistant *Acinetobacter baumannii* found that these AMPs showed potent antibacterial and anti-biofilm activity, synergism with conventional antibiotics, and most importantly, low cytotoxicity against human skin cells.[19]

Mechanisms of action of AMPs

Although the exact mechanism(s) by which AMPs exert their microbicidal activity has yet to be completely clarified, it is generally accepted that AMPs mainly target the cytoplasmic membrane by permeation and cell lysis activities. Studies have shown that the mode of action of AMPs are specifically based on their structural properties, such as sequence, size, cationic nature, hydrophobicity and amphipathicity.[20] Unlike conventional antibiotics which usually act by inhibition of cell wall synthesis or DNA, RNA and protein synthesis, most AMPs permeabilize microbial membranes, affecting the transmembrane potential and resulting in cell death.[21] The simplest models of membrane permeation by peptides involve the formation of membrane-spanning pores as illustrated in the barrel-stave pore model while the carpet model is the most commonly cited model of membrane destabilization by AMPs.[22] In addition to membrane permeabilization, AMP can result in neutralization or disaggregation of the lipopolysaccharide (LPS), a main endotoxin responsible for Gram-negative infections and collectively provide protection against sepsis.[23]

Alamethicin, a peptide antibiotic induces the formation of a unique transmembrane pore, which is similar to a barrel composed of helical peptides as staves (**barrel-stave model**).[24] Structural studies have shown that alamethicin adopts an α-helical configuration, attaches to, aggregates and inserts into oriented bilayers that are hydrated with water vapour.[25] Based on the experimental results obtained with dermaseptin to illustrate **carpet model**, peptides partition into acidic and zwitterionic membranes.[26] Peptides are then electrostatically attracted to the anionic phospholipid head groups at numerous sites covering the surface of the membrane in a carpet-like manner. At threshold concentrations of peptides, the self-oriented peptides form toroidal transient holes in the membrane, eventually leading to the formation of micelles.[26] In **toroidal pore model**, as exhibited by magainins, protegrins and melittin,[24,27] AMP helices insert into the membrane and induce the lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid head groups.[27] The interactions between the negatively-charged lipids and positively-charged peptides result in the formation of a continuous bend from the top to the bottom in the form of a toroidal hole.[23] Other models which account for membrane destabilization include the detergent model which explains the disintegration of the membrane with high concentrations of AMPs, the molecular shape model which describes AMP-membrane interactions and the resulting membrane morphologies[28]; the lipid clustering model in which AMPs induce separation of lipid components and resulting in the clustering of anionic lipids and formation of phase boundary defects among lipid domains[29]; the sinking raft model in which the detailed kinetic mechanism for AMP binding, association and insertion is formulated to explain the activity of AMP[30]; and the interfacial activity model in which the complex function of the hydrophobicity, amphipathicity and propensity of AMPs to induce membrane permeabilization is predicted.[31]

Studies have shown that AMPs and their combinations with antibiotics exhibits anti-biofilm activities. However, the mechanisms of inhibition and eradication of biofilms have not been reported. A possible mechanism could be the synergism of AMPs and antibiotics disrupt the biofilm matrix to allow AMPs to target the bacterial cells in the biofilm and promotion of dispersion of cells in the biofilm. Other possible mechanisms of action of AMPs in inhibiting biofilm formation could be interference of quorum sensing and inhibition of adhesion of bacterial cells on solid surfaces.

Potential of AMP as an anti-biofilm agent

AMPs have many of the attractive features of a novel antibiotic class, such as broad spectrum of activity, low incidence of bacterial resistance and specific mode of action which involves pore formation in the cytoplasmic membrane.[21] AMPs generally exhibit high stability in wide ranges of pH and temperature, properties that may be beneficial for scaled-up production and formulation into deliverable products. Due to their specific mode of actions, AMPs also exhibit low toxicity for eukaryotic cells, thus providing an opportunity for a wide therapeutic window. Low concentrations of AMPs which have demonstrated inhibitory and disruptive properties that eliminate even well-established biofilms have also been reported. Moreover, AMPs show synergy with classical antibiotics, neutralize endotoxin and are active in animal models.[32] Resistance to AMPs are relatively rare due to their attraction to the negatively-charged lipid bilayer structure of bacterial membranes.[33] Permeabilization and formation of pores within the cytoplasmic membranes, which is the prevalent mechanism of action, enables AMPs to act on slow-growing or even non-growing bacteria.[34] The killing kinetics of AMPs are also faster compared to most conventional antibiotics.[35] Further potential of AMPs include the ability to act at different stages of biofilm formation and with different mechanisms of action, such as down-regulation of QS,[17] killing of pre-formed biofilm, inhibition of biofilm formation and inhibition of adhesion.[36] AMPs are also often active against multidrug-resistant bacterial strains.[37] There are still limited reports of AMPs with the ability to inhibit the quorum sensing systems used in the different bacterial pathogens, although there is more understanding of the roles of QS in biofilm formation.

Most AMPs in pre-clinical and clinical trials today have been developed for topical applications. Examples of indications are catheter site infections, cystic fibrosis, acne and wound healing. Two AMPs, omiganan and pexiganan, have shown efficacy in Phase III clinical trials, but neither of them has been approved for clinical use. Pexiganan, which is a synthetic variant of magainin 2 and the best studied AMP in terms of drug development, was developed as a novel topical broad-spectrum antibiotic for the treatment of mild-to-moderate diabetic foot ulcer infections,[38] while omiganan was developed as a topical gel for prevention of catheter-associated infections.[39] A new class of antimicrobial peptide, called Selectively Targeted Antimicrobial Peptides (STAMPs) that has increased killing potency, selectivity and kinetics against targeted bacteria, have been developed.[40] Oritavancin, a semi-synthetic lipoglycopeptide in clinical development for the treatment of serious Gram-positive infections, exerts activity against methicillin-susceptible (MSSA), methicillin-resistant (MRSA), and vancomycin-resistant *S. aureus* (VRSA) with killing kinetics much faster than vancomycin.[41] The polymixins, a class of CAMPs has been used clinically as the last-line antibiotics to treat Gram-negative bacterial infections.[42]

Challenges to therapeutic use of AMP

Despite promising features of AMPs as potential therapeutics, there are limitations that need to be addressed for future development. AMPs display a significant reduction of their antimicrobial potency in the presence of biological fluids (for example, serum and saliva) compared to non-physiological conditions (for example, in phosphate buffer) due to the high concentrations of salt, anionic proteins and polysaccharides found in the biological fluids, and inactivation of AMPs by host and bacterial proteases during the course of infection.[43] Biofilms which contain bacterial DNA and the other polymers create a hydrated and charged environment surrounding the bacterial surface can prevent the access of cationic AMP LL-37.[5]

AMPs which are not of human origin may potentially cause damage to human cells. Melittin, an alkaline polypeptide composed of 26 amino-acid residues and the major component of European honeybee (*Apis mellifera*) venom, has been reported to be lytic to normal healthy cells, including erythrocytes.[44]

Poor physical—chemical properties, such as protein aggregation, particulate formation and reversible self-association contribute to increased viscosity and impede subcutaneous administration of high dose protein formulations with less than 1.5 mL volume limitation.[45] AMPs can also be susceptible to proteolytic degradation. In terms of production, AMPs are difficult and expensive to obtain in large quantities, due to the complex processes needed for their extraction, isolation and purification.[46]

Future considerations

Various attempts have been made to develop AMPs as innovative antimicrobials. However, to design them rationally is difficult because of the complex interaction of the peptides with membranes and with each other. Structural features that have been identified to be relevant for the microbicidal function of AMPs are the size, the sequence, the charge, the helicity, the overall hydrophobicity, the amphipathicity, and the angles subtended by hydrophobic and hydrophilic surfaces of the helical molecule.[32] Although promising AMPs have progressed to the pre-clinical and clinical stages of development as therapeutics, more studies are required to identify novel natural AMPs and new approaches to improve their activity, delivery and stability to increase the range of peptide therapeutics available in the clinic for broader applications.[47] Further focus into AMPs as biofilm prevention and eradication agents can involve investigations of AMPs that are enzymes or act like enzymes, such as deoxyribonuclease I and glycoside hydrolase dispersin B.[48] Limited data is available on novel AMPs with anti-biofilm properties, thus more investigations are also required to understand the precise mechanisms of action, particularly quenching of QS biofilm-promoting signals. It is also crucial to extend the studies on the dispersion effects of AMPs to already preformed biofilms and to developing biofilms. Synergism studies involving AMPs are also urgently needed to discover successful AMP—AMP or AMP-drug combinations to ensure minimal side effects of these antimicrobials.[16] Commercial-scale production platforms to synthesize AMPs are also urgently needed to overcome the high production cost, which remains the principal hurdle to overcome.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1. James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J, et al. Biofilms in chronic wounds. *Wound Repair Regen* 2008;**16**:37—44.
2. Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischaemia. *J Wound Care* 2010;**17**:145—55.
3. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;**415**:389—495.
4. Yeaman R, Yount NY. Mechanisms of antimicrobial peptide action and resistance. *Pharmacol Rev* 2003;**55**:27—55.
5. Nakatsuji T, Gallo RL. Antimicrobial peptides: old molecules with new ideas. *J Invest Dermatol* 2012;**132**:887—95.
6. Hancock REW, Lehrer R. Cationic peptides: a new source of antibiotics. *Trends Biotechnol* 1998;**16**:82—8.
7. Giuliani A, Pirri G, Nicoletto SF. Antimicrobial peptides: an overview of a promising class of therapeutics. *Cent Eur J Biol* 2007;**2**:1—33.
8. Brandenburg L, Merres J, Albrecht L, Varoga D, Pufe T. Antimicrobial peptides: multifunctional drugs for different applications. *Polymers* 2012;**4**:539—60.
9. Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: more than just microbicidal. *Trends Immun* 2002;**23**:291—6.
10. Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol* 2009;**30**:131—41.
11. Zhou Y, Peng Y. Synergistic effect of clinically used antibiotics and peptide antibiotics against Gram-positive and Gram-negative bacteria. *Exp Ther Med* 2013;**6**:1000—4.
12. 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.
13. Todorov SD, Wachsman MB, Knoetze H, Meincken M, Dicks LMT. An antibacterial and antiviral peptide produced by *Enterococcus mundtii* ST4V isolated from soya beans. *Int J Antimicrob Agents* 2005;**25**:508—13.
14. Dean SN, Bishop BM, van Hoek ML. Natural and synthetic cathelicidin peptides with anti-microbial and anti-biofilm activity against *Staphylococcus aureus*. *BMC Microbiol* 2011;**11**:114—25.
15. 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—10.
16. Jorge P, Lourenço A, Pereira MO. New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. *Biofouling* 2012;**26**:1033—61.
17. Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infect Immun* 2008;**76**:4176—82.
18. Hell E, Giske CG, Nelson A, Römling U, Marchini G. Human cathelicidin peptide LL-37 inhibits both attachment capability and biofilm formation of *Staphylococcus epidermidis*. *Lett Appl Microbiol* 2010;**50**:211—5.
19. Gopal R, Kim YG, Lee JH, Lee SK, Chae JD, Son BK, et al. Synergistic effects and antibiofilm properties of chimeric peptides against multidrug-resistant *Acinetobacter baumannii* strains. *Antimicrob Agents Chemother* 2014;**58**:1622—9.
20. Bhattacharjya S, Ramamoorthy A. Multifunctional host defense peptides: functional and mechanistic insights from NMR structures of potent antimicrobial peptides. *FEBS J* 2009;**276**:6465—73.
21. Hancock REW, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 2006;**24**:1551—7.
22. Wimley WC, Hristova K. Antimicrobial peptides: successes, challenges and unanswered questions. *J Membr Biol* 2011;**239**:27—34.
23. Kaconis Y, Kowalski I, Howe J, Brauser A, Richter W, Razquin-Olazarán I, et al. Biophysical mechanisms of endotoxin neutralization by cationic amphiphilic peptides. *Biophys J* 2011;**100**:2652—61.
24. Yang L, Harroun TA, Weiss TM, Ding L, Huang HW. Barrel-stave model or toroidal model? A case study on melittin pores. *Biophys J* 2001;**81**:1475—85.
25. Spaar A, Munster C, Salditt T. Conformation of peptides in lipid membranes studied by X-ray grazing incidence scattering. *Biophys J* 2004;**87**:396—407.
26. Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y. Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes. *Biochemistry* 1992;**31**:12416—23.
27. Matsuzaki K, Murase O, Fujii N, Miyajima K. An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. *Biochemistry* 1996;**35**:11361—8.
28. Bechinger B, Lohner K. Detergent-like actions of linear amphipathic cationic antimicrobial peptides. *Biochim Biophys Acta* 2006;**1758**:1529—39.
29. Epand RM, Epand RF. Lipid domains in bacterial membranes and the action of antimicrobial agents. *Biochim Biophys Acta* 2009;**1788**:289—94.
30. Pokorny A, Birkbeck TH, Almeida PFF. Mechanism and kinetics of delta-lysin interaction with phospholipid vesicles. *Biochemistry* 2002;**41**:11044—56.
31. Rathinakumar R, Wimley WC. Biomolecular engineering by combinatorial design and high-throughput screening: small, soluble peptides that permeabilize membranes. *J Am Chem Soc* 2008;**130**:9849—58.
32. Koczulla AR, Bals R. Antimicrobial peptides: current status and therapeutic potential. *Drugs* 2003;**63**:389—406.
33. Bahar AA, Ren D. Antimicrobial peptides. *Pharmaceuticals* 2013;**6**:1543—75.
34. Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M. Use of antimicrobial peptides against microbial biofilms: advantages and limits. *Curr Med Chem* 2011;**18**:256—79.
35. Maisetta G, Batoni G, Esin S, Florio W, Bottai D, Favilli F, et al. *In vitro* bactericidal activity of human beta-defensin 3 against multidrug-resistant nosocomial strains. *Antimicrob Agents Chemother* 2006;**50**:806—9.
36. Arslan SY, Leung KP, Wu CD. The effect of lactoferrin on oral bacterial attachment. *Oral Microbiol Immunol* 2009;**24**:411—6.
37. Mangoni ML, Maisetta G, Di Luca M, Gaddi LM, Esin S, Florio W, et al. Comparative analysis of the bactericidal activities of amphibian peptide analogues against multidrug-resistant nosocomial bacterial strains. *Antimicrob Agents Chemother* 2008;**52**:85—91.
38. Seo MD, Won HS, Kim JH, Mishig-Ochir T, Lee BJ. Antimicrobial peptides for therapeutic applications: a review. *Molecules* 2012;**17**:12276—86.
39. Fritsche TR, Rhomberg PR, Sader HS, Jones RN. Antimicrobial activity of omiganan pentahydrochloride against contemporary fungal pathogens responsible for catheter-

associated infections. *Antimicrob Agents Chemother* 2008; **52**:1187—9.
40. Eckert R, Qi F, Yarbrough DK, He J, Anderson MH, Shi W. Adding selectivity to antimicrobial peptides: rational design of a multidomain peptide against *Pseudomonas* spp. *Antimicrob Agents Chemother* 2006;**50**:1480—8.
41. Allen NE, Nicas TI. Mechanism of action of oritavancin and related glycopeptide antibiotics. *FEMS Microbiol Rev* 2003;**26**:511—32.
42. Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin b for the treatment of multidrug-resistant pathogens: a critical review. *J Antimicrob Chemother* 2007;**60**:1206—15.
43. 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.
44. Tosteson MT, Holmes SJ, Razin M, Tosteson DC. Melittin lysis of red cells. *J Membr Biol* 1985;**87**:35—44.
45. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. *J Pharm Sci* 2004;**93**: 1390—402.
46. Bradshaw J. Cationic antimicrobial peptides: issues for potential clinical use. *BioDrugs* 2003;**17**:233—40.
47. Easton DM, Nijnik A, Mayer ML, Hancock RE. Potential of immunomodulatory host defense peptides as novel anti-infectives. *Trends Biotechnol* 2009;**27**:582—90.
48. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med* 2014;**3**, a010306.