Evaluation of antimicrobial peptide LL-37 for treatment of Staphylococcus aureus biofilm on titanium plate

Jiantong Wei, MD\textsuperscript{a,b}, Xuepeng Cao, BS\textsuperscript{a}, Jun Qian, MD\textsuperscript{a,b,*}, Zhixia Liu, BS\textsuperscript{a}, Xulong Wang, BS\textsuperscript{a,b}, Qinliuye Su, BS\textsuperscript{a}, Yongpin Wang, MD, PhD\textsuperscript{c}, Ruimin Xie, MD\textsuperscript{c}, Xiang Li, MD\textsuperscript{c}

Abstract
The antimicrobial peptide LL-37 belongs to the cathelicidin family and is one of the few human bactericidal peptides with potent antistaphylococcal activity. Staphylococcus aureus is one of the main infection bacteria in orthopedic implant therapy. Biofilm formation after bacterial infection brings more and more severe test for clinical antiinfection treatment.

However, there are few studies on LL-37 in S. aureus infection of prosthesis. In this work, addition to research the antibacterial activity and the inhibitory effect on bacterial adhesion of LL-37, an in vitro model of S. aureus biofilm formation on titanium alloy surface was established to observe the inhibitory effect of LL-37.

The results showed that LL-37 has a strong antibacterial effect on S. aureus in vitro, and the minimum inhibitory concentration (MIC) is about 0.62 \(\mu\)M. Moreover, LL-37 has significant impact on the adhesion of S. aureus when the concentration \(\geq 0.16 \mu\text{M}\) and significant anti-staphylococcal biofilm effects on static biofilm models at the concentration of 0.31 to 10 \(\mu\text{M}\). Additionally, LL-37 at 5 \(\mu\text{M}\) had a significant destructive effect on S. aureus biofilm (\(P < .05\)) that formed on the titanium alloy surface.

This study further confirmed the role of LL-37 in the process of S. aureus infection, including antimicrobial activities, inhibition of bacterial adhesion, and inhibition of mature biofilm. LL-37 can significantly destroy the stable biofilm structure on the titanium alloy surface in vitro, which may provide a new way for refractory infection caused by S. aureus in titanium alloy prosthetic infection.

Abbreviations: MIC = minimum inhibitory concentration, PBS = phosphate-buffered saline, TSB = tryptic soy broth.

Keywords: antimicrobial peptide, biofilm, LL-37, Staphylococcus aureus, titanium plate

1. Introduction
Antimicrobial peptides are a class of active antigens induced by the biological immune system, which can resist the infection of a variety of pathogens and are an important part of the innate immune system of multicellular organisms.\(^1\) Compared with traditional antibiotics, antimicrobial peptides have the advantages of broad antibacterial spectrum and faster bactericidal rates.\(^2,3\) With the emergence of antibiotic-resistant strains in large numbers, the application of antimicrobial peptides as a new antibacterial agent in clinical treatment has aroused many scholars’ interest, also provided a new idea and means for the treatment of clinical infection.\(^4\) Clinically, 80% of human bacterial infections are in the form of biofilm. Bacterial biofilm appears in the form of bacterial community, showing high resistance to antibiotics.\(^5\) According to statistics, bacteria in biofilms can be 100 to 1000 times more resistant to antibiotics than their planktonic state.\(^6\) Bacterial biofilm is an organized group of bacteria formed by attaching multiple bacteria to non-biological or biological surfaces, secreting a polymer matrix and wrapping itself in it. Once bacteria are colonized on the surface of human life tissues (heart, lung, intestine, urethra, etc.) or medical biomaterials (artificial heart valves, artificial joints, implanted catheters, etc.), bacterial biofilm infection may be caused.\(^7\) As a new antibacterial agent, antimicrobial peptides play a unique advantage in the process of inhibiting and killing bacterial biofilm.\(^8\)

Antimicrobial peptide LL-37 is the only member of cathelicidins family found in human body. LL-37 is expressed and secreted in a variety of epithelial cells, immune cells, body fluids, trauma secretions, etc. in the human body. It can exert antimicrobial activity, participate in immune regulation of the body, and promote wound repair. It is an active small molecule peptide with multiple functions.\(^9\) Studies have shown that LL-37 has a broad antibacterial spectrum, such as antibacterial, antifungal, antiviral, antiprotozoal and other antimicrobial
effects.[17–20] The antibacterial activity of LL-37 is higher than that of traditional antibiotics, and its minimum inhibitory concentration (MIC) is similar to that of fluoroquinolones such as ofloxacin and ciprofloxacin, but higher than that of norfloxacin and enoxacin.[21] Existing studies have shown that LL-37, as an endogenous antibiotic not only has a good sterilization effect on planktonic Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and other pathogens, also has inhibitory activity against S. aureus, Staphylococcus epidermidis, P. aeruginosa, Acinetobacter baumannii in the form of biofilm.[22–25]

In orthopedic implants, titanium alloy (Ti6Al4V) materials are widely used. Biomedical titanium alloy materials are a class of functional structural materials applied in biomedical engineering, often applied in the production and manufacturing of surgical implants and orthopedic devices. Titanium alloy medical equipment products such as artificial joints, dental implants and vascular stents are used for clinical diagnosis, treatment, repair, replacement of human tissues or organs, or to enhance the function of human tissues or organs, and their role cannot be replaced by drugs. Studies have shown that the biofilm formed by S. aureus is easy to adhere to the surface of metal implants such as titanium alloys, while S. epidermidis is easy to adhere to the surface of polymers.[26] Previous studies in the literature regarding the efficacy of LL-37 against preformed biofilms vary. Some studies suggest that LL-37 does not disrupt preformed biofilms, inhibit bacterial attachment, or prevent early biofilm formation.[27,28] In contrast, other studies have demonstrated that LL-37 can disrupt 24 and 48 hours mature S. aureus biofilms.[29] Therefore, in this study, a bacterial biofilm of S. aureus in vitro was constructed on the surface of titanium alloy to simulate the infection related to orthopedic implants.

In this study, the in vitro antibacterial activity of LL-37 on S. aureus was measured first, and the effect of LL-37 on the adhesion of S. aureus was determined. Then, an in vitro static model of S. aureus biofilm, a common pathogen of orthopedic implants, was established to further observe the damaging effect of LL-37 on the S. aureus biofilm and the S. aureus biofilm formed on the surface of titanium alloy. The purpose of this study was to clarify the role of LL-37 in the process of S. aureus infection, provide a theoretical basis for clinical treatment of S. aureus infection of orthopedic implants.

2. Materials and methods

2.1. Materials

Staphylococcus aureus subsp. aureus (ATCC 6538P) obtained from Guangdong Microbial Culture Collection center (GDMCC). The human antimicrobial peptide LL-37 (LLGDFKRKSKEKIGKEFKRIVQRKDFLRNLVPRTES), with 98.67% purity, was purchased from China Peptides Co., Ltd. (Shanghai, China). Titanium plate (Ti6A14V, diameter 1.0 cm, thickness 3 mm) provided and processed by Tianjin Jinxingda Industrial Co., Ltd. (Tianjin, China). Tryptic soy broth (TSB), phosphate-buffered saline (PBS) and other reagents were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The equipment used in this study includes a constant temperature incubator (BPJX-400-1, Shandong Brocade Biological Industry Co., Ltd., Jinan, China), a Motic Digital BA310 Biological Light Microscope (Motic China Group Co., Ltd, Xiamen, China) an Olympus Fluoview FV1000 laser scanning confocal microscope (Olympus Corp., Tokyo, Japan), an ultraviolet and visible spectrophotometer (UV-5100 manufactured by Shanghai Kunke Instrument Equipment Co., Ltd.) and a RT-6100 microplate reader (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). This study does not involve human or animal participants, hence ethical approval is not required.

2.2. Preparation of bacterial suspension

Firstly, 0.3 mL of normal saline was injected into the opened strain ampoule by pipetting to dissolve the freeze-dried strain in the ampoule into a bacterial suspension. Aspirated the above suspension and inoculated to a sheep blood agar plate was divided into 3 sectors, the plates were inverted and incubated at 37°C for 24 hours. Then picked a single colony and inoculated it in 5 mL TSB and placed it in a 37°C constant temperature incubator, shook culture, and the oscillation frequency was set to 180 rpm. After shaking for 16 hours, an ultraviolet spectrophotometer used to adjust the standard bacterial solution with a concentration of about 1.5 × 10⁸ CFU/mL (OD450 = 0.29).

2.3. Determine the minimum inhibitory concentration of LL-37

The MIC was determined according to the NCCLS standard. Briefly, in 96-well microtiter plates, added 180 μL TSB to the first well, each of 2 to 11 wells added 100 μL. Then added 20 μL of LL-37 supernatant (20 μM) to the first well, mixed and aspirated 100 μL into the second well, the same method was use to dilute to the tenth well, discard 100 μL. Then 100 μL of standard bacterial solution was added to each well. The concentration of LL-37 in each well is 10, 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, 0.04, and 0.02 μM, respectively. The negative control was 100 μL of normal saline and all experiments were repeated 3 times. After incubating at 37°C for 24 hours, the OD value at 450 nm of each well was measured with microplate reader.

2.4. Determination the effect of LL-37 on the adhesion of S. aureus

Inoculated 100 μL of standard bacterial solution in a 96-well plate and incubated at 37°C for 1, 2, and 4 hours respectively. Aspirated and discarded the medium, then gently rinsed with PBS 3 times to wash away non-adherent bacteria. Added LL-37 in 2-fold serial dilutions (10, 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, 0.04, and 0.02 μM) to wells 1 to 10. The negative control was added the same volume of normal saline and incubated continuously at 37°C for 24 hours. Then a microplate reader was used to detect the absorbance value (OD450) of each well. The experiment was repeated 3 times, 8 replicate holes were set for each drug concentration, and the average value was taken.

2.5. Establishment of an in vitro bacterial biofilm model

Pipetted 10 μL of standard bacterial solution into a 24-well plate, added 1 mL TSB to each well, cultured at 37°C, replaced the culture medium once every 24 hours, and cultured for 4 consecutive days. The culture medium supernatant was stained with crystal violet, and the 24-well plate was gently washed with sterile PBS 3 times to remove the floating S. aureus. After air-drying in the ultra-clean workbench, stained with 1% Fuchsins for 30 seconds, rinsed gently with PBS solution 3 times, let it air dry.
Finally, the images were recorded using the microphotography system (BA310 Digital, McAudi Industrial Group Co., LTD.).

2.6. Destructive effect of LL-37 on S. aureus biofilm

Pipetted 10 μL of standard bacterial solution into a 24-well plate, and 1 mL TSB was added to each well. After autoclaving the titanium alloy plate, place it in a 24-well plate containing the above-mentioned bacterial solution, and incubated at 37°C for 1 week, and replaced the culture solution every day. One week later, they were randomly divided into experimental group and control group. The experimental group was added with 5 μM LL-37, and the control group was added with the same volume of broth medium. After incubating at 37°C for 24 hours, rinsed with PBS several times and fixed with 2.5% glutaraldehyde solution at 4°C overnight. 30%, 50%, 70%, 90%, 100% ethanol gradient dehydration was used for 10 minutes, and 100% ethanol was used twice. The sample is placed in a desiccator, and the dried specimen is placed in a high-vacuum evaporator, to be tested after gold spraying by an ion sprayer. Finally, an Olympus Fluo View FV1000 laser scanning confocal microscope was used to observe the ultrastructure of the bacterial biofilm on the titanium plate.

The above experiment was repeated without adding titanium alloy plate to observe the ultrastructure of the S. aureus biofilm in vitro. In addition, the quantitative detection of S. aureus biofilm by crystal violet staining method. The specific method is as follows: added 200 μL of standard bacterial solution to the 96-well plate, incubated at 37°C for 1 week, and replaced the culture solution every day. One week later, different concentrations of LL-37 (10, 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, 0.04, and 0.02 μM) were added to wells 1 to 10, and the control group was added with the same volume of TSB. Standing at 37°C for 24 hours, rinsed the 96-well plate with PBS. After air drying, added 200 μL of 1% crystal violet, dyed for 30 minutes at room temperature, then rinsed the dye solution with distilled water. After natural air drying, added 95% ethanol and stand for 10 minutes. 95% ethanol was used as a blank control, and the absorbance value at 450 nm (OD450) was measured with microplate reader.

2.7. Statistical analysis

The results were analyzed using SPSS17.0 statistical software, and all data were expressed as mean ± standard deviation. An analysis of variance (ANOVA) followed by Tukey’s honestly significant difference (HSD) test was used to determine significance between groups, and a P value of <.05 was considered statistically significant.

3. Results

3.1. Determine the minimum inhibitory concentration of LL-37

The antibacterial activity of LL-37 against S. aureus was obtained by two-fold micro dilution method at the concentration of 10, 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, 0.04, and 0.02 μM (Fig. 1). After incubation, the MIC was read as the lowest concentration of antimicrobial agent that visibly inhibited bacterial growth. The results showed that the MIC of LL-37 against S. aureus was about 0.62 μM.

3.2. The effect of LL-37 on the adhesion of S. aureus

After culturing S. aureus for 1, 2, and 4 hours, different concentrations of LL-37 were added, and negative controls were set. The absorbance (OD450) was measured with a spectrophotometer. The results show (Fig. 2A, B, and C) that different LL-37 concentrations have a significant impact on the adhesion of S. aureus. Compared with the negative control group, when the LL-37 concentration at 0.16 μM, the amount of bacteria attached was significantly reduced (P < 0.05). That is, LL-37 can inhibit the initial attachment behavior of S. aureus at 1/4 MIC concentration, and the inhibitory effect shows a dose enhancement. However, the different culture time of S. aureus (after 1, 2, and 4 hours) did not have a significant effect.

3.3. The static bacterial biofilm model in vitro

To establish an in vitro static biofilm model, the standard bacterial solution was added to the 24-well plate and incubated at 37°C for 4 consecutive days. The results show that S. aureus could form mature biofilm after 4 days of continuous cultivation. After fuchsin staining, the bottom of 24 well plate was covered with red dense substance. Under the microscope, lots of bacteria can be seen to grow and form membranous colonies (Fig. 3A). The planktonic S. aureus in the culture medium was observed under high power microscope after Gram staining (Fig. 3B). The results showed that S. aureus formed stable biofilm in vitro under the current condition, this static biofilm model could be used for follow-up study.

3.4. Anti-staphylococcal biofilm effects of LL-37

In order to observe the anti-staphylococcal biofilm effects of LL-37, we prepared static biofilm models in vitro and titanium alloy carrier biofilm models, and 5 μM LL-37 was used to experimental group. Scanning electron microscope observation revealed that the control group was significantly different from the LL-37 group, as shown in Fig 4. The mature biofilm structure of the control group is still intact, and the bacteria are densely arranged (Fig. 4A, B, E, and F). In the LL-37 treatment group, the arrangement of the biofilm was loose, the total amount of mature plaque was significantly reduced, and only a few intact bacteria were seen (Fig. 4C, D, G, and H). From the above results, we can...
Figure 2. The effect of LL-37 on the adhesion of S. aureus. After culturing S. aureus for 1 (A), 2 (B), 4 (C) hours, inhibitory activity of LL-37 on S. aureus biofilm (D). Statistical analysis was calculated by Tukey’s honestly significant difference (HSD) test. N = 3 independent experiments, 8 replicates in each (* P < .05, ** P < .01; *** P < .001).

Figure 3. Micrographs of S. aureus and its biofilm. (A) S. aureus biofilm formed in vitro, stained with 1% acid fuchsin. Magnifications: 40×. (B) Planktonic S. aureus in the medium, stained with 1% crystal violet solution. Magnifications: 1000×.
see that LLC at a concentration of 5 μM exert a strong inhibitory effect on the mature biofilm of S. aureus (Fig. 4C, D). Moreover, the scanning electron microscope observation results show that LLC has a significant anti-staphylococcal biofilm effects on the titanium alloy surface (Fig. 4G, H). As shown in Figure 4G, the structure of biofilm and mature plaque almost
disappeared, and only a small number of damaged bacteria were seen, while the complete biofilm structure can be observed under the control group (Fig. 4H).

The analysis results of crystal violet staining method showed that compared with the control group, the total amount of biofilm in the different concentrations of LL-37 treatment group decreased to varying degrees, and the 1/4 MIC concentration group had a statistically significant difference (P < .05) (Fig. 2D). This indicates that LL-37 can affect the formed biofilm at the MIC concentration and exhibit a dose-dependent enhancement.

4. Discussion

The results showed that LL-37 had strong antibacterial activity in a dose-dependent manner at nanomolar concentrations in vitro. This result is consistent with the research results reported previously. Noore et al found that LL-37 was effective in killing extracellular S. aureus at nanomolar concentrations, while lactoferrin B was effective at micromolar concentrations and doxycycline and cefazolin at millimolar concentrations. LL-37 was found to exhibit over 90% killing efficacy at as low as 250 nM, over 99% at 500 nM, and 100% at 3.0 μM.[10] In addition, related studies have shown that LL-37 has higher antibacterial activity than commonly used antibiotics, and it is not easy to develop drug resistance[22,30,31] These studies indicate that LL-37 is an ideal non-antibiotic bacteriostatic agent.

Surface attachment is regarded as the first step for biofilm formation. In previous studies, the experimental results of LL-37 effect on the adhesion of S. aureus were different. Mishra et al found that LL-37 in the concentration range from 3.1 to 25 μM was unable to inhibit the attachment of S. aureus USA300.[27] Luo et al used the crystal violet assay biofilm biomass showed that LL-37 had significant efficacy in preventing biofilm formation by S. aureus but was unable to inhibit early biofilms of S. aureus.[28] Our experimental results show that in the presence of LL-37, the initial attachment rate of S. aureus is significantly reduced, which directly reduces the number of initially attached bacteria on the surface of the carrier. LL-37 played an important intervening role in the first step of biofilm formation.

In recent years, the effect of LL-37 on the S. aureus biofilm has attracted lots of attention from researchers. Related research shows that LL-37 can effectively inhibit S. aureus biofilm.[14,22,32,33] Luo et al believe that LL-37 can prevent the formation of biofilms, but has no effect on the formed biofilms.[28] Mishra et al observed that LL-37 was unable to inhibit bacterial attachment or disrupt preformed biofilm.[27] In our research, the inhibitory effect of LL-37 on the formed biofilm was verified. In addition, we also found that LL-37 has obvious inhibitory activity on the formed biofilm on the titanium alloy surface.

5. Conclusion

This study further confirmed the role of antimicrobial peptide LL-37 in the process of S. aureus infection, including antimicrobial activities, inhibition of bacterial adhesion, and inhibition of mature biofilm. Briefly, LL-37 in vitro on S. aureus has a strong antibacterial effect, and the MIC is about 0.62 μM. When the concentration of LL-37 is 0.16 μM, it has a significant impact on the adhesion of S. aureus and prevents the formation of bacterial biofilm. In addition, LL-37 can significantly destroy the mature biofilm structure and the stable biofilm structure on the titanium alloy surface. The biofilm in this study is a model in vitro, which has a certain gap with the infection biofilm of clinical cases. In the future, a more mature biofilm model should be established to simulate clinical infection cases.

Author contributions

Conceptualization: Jun Qian and Yongpin Wang.
Data curation: Zhixia Liu, Xulong Wang, Qinliuye Su.
Funding acquisition: Jiantong Wei.
Investigation: Xuepeng Cao, Yongpin Wang, Xiang Li.
Resources: Jiantong Wei.
Software: Ruimin Xie.
Writing – original draft: Xuepeng Cao.
Writing – review & editing: Jiantong Wei, Xuepeng Cao, Jun Qian.

References

[1] Bahar AA, Ren D. Antimicrobial peptides. Pharmaceuticals 2013;6: 1543–75.
[2] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389–95.
[3] Gong Z, Pei X, Ren S, et al. Identification and rational design of a novel antibacterial peptide dermaseptin-AC from the skin secretion of the red-eyed tree frog Agalychnis callidryas. Antibiotics 2020;9:243.
[4] Wennel M, Chiriac AI, Otto A, Zwyetick D, Bandow JE. Small cationic antimicrobial peptides delocalize peripheral membrane proteins. Proc Natl Acad Sci U S A 2014;111:E1409–18.
[5] Raheem N, Strauss SK. Mechanisms of action for antimicrobial peptides with antibacterial and antibiofilm functions. Front Microbiol 2019;10:2866.
[6] Straus RE, Hancock REW. Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: Comparison with cationic antimicrobial peptides and lipopeptides. Biochim Biophys Acta 2006;1758:1213–23.
[7] Haney EF, Nguyen LT, Schibli DJ, Vogel HJ. Design of a novel tryptophan-rich membrane-active antimicrobial peptide from the membrane-proximal region of the HIV glycoprotein, gp41. Beilstein J Org Chem 2012;8:1172–84.
[8] Haney EF, Hancock REW. Peptide design for antimicrobial and immunomodulatory applications. Biopolymers 2013;100:572–83.
[9] Uhlig T, Kyprianou T, Martinelli FG, et al. The emergence of peptides in the pharmaceutical business: from exploration to exploitation. Eupa Open Proteomics 2014;4:58–69.
[10] Chai JO, Yoo JL, Yoo JS, Chung HS, Chung GT. Investigation of biofilm formation and its association with the molecular and clinical characteristics of methicillin-resistant Staphylococcus aureus. Osong Public Health Res Perspect 2013;4:225–32.
[11] Heshy N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010;35:322–32.
[12] Mah TF, O’Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001;9:34–9.
[13] Sagmur R, Stdenis M, Ferris W, et al. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrob Agents Chemother 2006;50:65–71.
[14] Ding J, Wang YZ, Shen J, Zhu JY, Jin XB. Effect of human antimicrobial peptide LL-37 on methicillin-resistant Staphylococcus aureus biofilms. J Guangdong Pharm Univ 2016;4:498–502.
[15] Zhang LJ, Guerrero-Juarez CF, Hata T, et al. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. Science 2015;347:67–71.
[16] Durr UHN, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochim Biophys Acta 2006;1758:1408–25.
[17] Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Anti-microb Agents Chemother 1998;42:2206–14.
[18] Larrick JW, Hirata M, Zhong J, Wright SC. Anti-microbial activity of human CAP18 peptides. Immunotechnology 1995;1:65–72.
[19] Johansson J. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. J Biol Chem 1998;273:3718–24.

[20] Tanaka D, Miyasaki KT, Lehrrer RI. Sensitivity of Actinobacillus actinomycetemcomitans and Capnocytophaga spp. to the bactericidal action of LL-37: a cathelicidin found in human leukocytes and epithelium. Oral Microbiol Immunol 2010;15:226–31.

[21] Dorschner RA, Pestonjamasp VK, Tamakwala S, et al. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. J Invest Dermatol 2001;117:91–7.

[22] Kang J, Dietz MJ, Li B. Antimicrobial peptide LL-37 is bactericidal against Staphylococcus aureus biofilms. Plos One 2019;14:e0216676.

[23] Hell E, Giske CG, Nelson A, Romling U, Marchini G. Human cathelicidin peptide LL37 inhibits both attachment capability and biofilm formation of Staphylococcus epidermidis. Lett Appl Microbiol 2010;50:211–5.

[24] Overhage J, Campisano A, Bains M, Torfs ECW, Hancock REW. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 2008;76:4176–82.

[25] Shi P, Gao Y, Lu Z, Yang L. Effect of antibacterial peptide LL-37 on the integrity of Acinetobacter baumannii biofilm. Nan Fang Yi Ke Da Xue Bao 2014;34:426–9.

[26] Gupta A, Bastiaampillai T, Adams M, Nelson A, Nance M. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 1987;237:1588–95.

[27] Misra B, Golla RM, Lau K, Lushnikova T, Wang G. Anti-staphylococcal biofilm effects of human cathelicidin peptides. Acs Med Chem Lett 2015;7:117–21.

[28] Luo Y, Mclean DTF, Linden GJ, Mcauley DF, Ronan MM, Lundy FT. The naturally occurring host defense peptide, LL-37, and its truncated mimetics KE-18 and KR-12 have selected biocidal and antibiofilm activities against Candida albicans, Staphylococcus aureus, and Escherichia coli in vitro. Front Microbiol 2017;8:544.

[29] Mohamed MF, Abdelkhalek A, Seleem MN. Evaluation of short synthetic antimicrobial peptides for treatment of drug-resistant and intracellular Staphylococcus aureus. Sci Rep 2016;6:29707.

[30] Noore J, Noore A, Li B. Cationic antimicrobial peptide LL-37 is effective against both extra- and intracellular Staphylococcus aureus. Antimicrob Agents Chemother 2013;57:1283–90.

[31] Limoli DH, Rockel, et al. Cationic antimicrobial peptides promote microbial mutagenesis and pathoadaptation in chronic infections. Plos Pathog 2014;10:e1004083.

[32] Haisma EM, De Breij A, Chan H, et al. LL-37-derived peptides eradicate multidrug-resistant Staphylococcus aureus from thermally wounded human skin equivalents. Antimicob Agents Chemother 2014;58:4411–9.

[33] 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.