# The battle against antimicrobial resistant bacterial infections: next stage development of antimicrobial peptides

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# Summary and general discussion

## 1. Summary

Bacterial infections are becoming harder to treat with current antibiotics, due to antimicrobial resistance [1, 2]. In addition, treatment of chronic bacterial infections is further hampered by biofilm-formation and development of persister cells [1-4]. Simultaneously, the advancement of novel antibiotics or antibiotic classes has declined [1, 5]. These developments illustrate the urgent need for novel antibacterial agents. Antimicrobial peptides (AMPs) may fulfill this role. AMPs are relatively short (10-60 amino acids), often cationic, peptides and are part of the defense mechanism against bacteria in a broad range of organisms [6, 7]. Importantly, most AMPs have a distinct mechanism of action compared to current antibiotics and are capable of quickly killing a broad range of bacteria via destabilization and perforation of the bacterial plasma membrane [8]. Therefore, the risk of resistance development by bacteria in response to AMPs is relatively low [9]. However, to become suitable for clinical application, AMPs require optimization due to limitations regarding their cationic peptidic nature, i.e. limited selectivity index as a result of cytotoxicity towards mammalian cells, short half-life, limited stability and bioavailability, and low tissue penetration [10-12]. This thesis describes three strategies for optimization of our lead AMP SAAP-148 to combat bacterial infections: i) chemical lead-optimization, ii) combination therapy with other antimicrobial agents and iii) innovative AMP delivery systems. The latter strategy was also applied to another promising AMP, the snake cathelicidin Ab-Cath. A comprehensive introduction to this thesis is described in **chapter 1**.

The objectives of this thesis were:

1. To evaluate a library of synthetic variants of SAAP-148 for their *in vitro* antibacterial, immunomodulatory and cytotoxic activities.
2. To investigate the potential of SAAP-148 when used in combination with the novel antibiotic halicin.
3. To review the literature on lipid and polymeric AMP delivery systems and coatings and to explore candidate formulations to treat bacterial skin wound infections.
4. To develop hyaluronic acid-based nanogels incorporating SAAP-148 or snake cathelicidin Ab-Cath to improve their selectivity indices for cutaneous application on infected skin wounds.

The main findings of this thesis contribute to the development of AMPs as therapeutics to combat bacterial infections. Firstly, **chapter 2** described the chemical lead-optimization of SAAP-148 by chemical conjugation to short polyethylene glycol (PEG) groups of various lengths. It was shown that C-terminal PEGylation of SAAP-148 was most favorable for improving SAAP-148's selectivity index, by maintaining antimicrobial activities to AMR *S. aureus* and *E. coli* and simultaneously

reducing cytotoxicity to human erythrocytes. Importantly, C-terminal PEGylation enhanced the pro-inflammatory immunomodulatory activities of SAAP-148 towards cells involved in innate immunity, i.e. neutrophils, macrophages and dendritic cells, with SAAP-148-$PEG_{27}$ being most effective. In addition, it was found that the length of the PEG group, the conjugation position and the sequence of the peptide all contributed to the enhanced activities. Secondly, **chapter 3** compared the activities of SAAP-148 and novel antibiotic halicin when used alone or in combination. It was shown that combinations of SAAP-148 and halicin act synergistically against planktonic bacteria of specific AMR *S. aureus* and *E. coli* strains. Most importantly, these favorable interactions were confirmed in an *S. aureus* biofilm model on silicone disks that mimic synthetic materials, such as intravenous or urinary tract catheters, and in clinically relevant 3D models for *S. aureus* skin wound infections and *E. coli* bladder infections. Thirdly, recent literature on formulation of AMPs in lipid and polymeric drug delivery systems (DDSs) and coatings for the prevention and treatment of bacterial infections was reviewed in **chapter 4**. This chapter described promising *in vitro* and a limited amount of *in vivo* results for nano-sized DDSs and coatings that assist delivery of AMPs intracellularly and into bacterial biofilms. Based on the information available, we provided an overview of the requirements and recommended DDSs for major hard-to-treat bacterial infections. In addition, major challenges were pinpointed that have to be overcome for successful implementation of AMP DDSs and coatings in the clinic. Fourthly, development of a DDS composed of hyaluronic acid-based nanogels incorporating snake cathelicidin Ab-Cath for cutaneous application was described in **chapter 5**. It was shown that these nanogels allow for efficient encapsulation of Ab-Cath, harboring excellent physicochemical and functional properties. Importantly, a successful lyophilization method was developed that allowed long term storage and concentration of the AMP DDS. Furthermore, the lyophilized and redispersed Ab-Cath-loaded nanogels improved the peptide's selectivity index by maintaining antimicrobial activities against AMR *S. aureus*, *A. baumannii* and *E. coli* and reducing cytotoxic activities against human primary skin fibroblasts. Finally, the same DDS and lyophilization strategy was used to develop SAAP-148-loaded nanogels, which is described in **chapter 6**. Likewise, lyophilized and redispersed SAAP-148-loaded nanogels improved the peptide's selectivity index by maintaining antimicrobial activities against AMR *S. aureus* and *A. baumannii* and reducing cytotoxic activities against human erythrocytes, human primary skin fibroblasts and Ker-CT keratinocytes. Importantly, this improved selectivity was confirmed in a clinically relevant 3D human epidermal model colonized with AMR *S. aureus* or *A. baummannii*. Below, the findings of this thesis are placed in a broader perspective with added recommendations and future perspectives to promote AMP development and to introduce AMPs to the clinic as soon as possible.

## 2. General design of screening methods for novel antibacterial agents

### 2.1. Antimicrobial resistance development, anti-biofilm and anti-persister activities and PK/PD properties as main parameters for antibiotic drug discovery

Based on the above mentioned major threats for effective treatment of bacterial infections, it is of most importance to develop novel antibiotics and/or other alternatives that do not quickly induce resistance and that are effective against biofilm-residing bacteria and persister cells. Currently, lead optimization of hits from high-throughput screenings do not always take these parameters into account, but focus mainly on effects towards planktonic bacteria, i.e. minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing [13, 14]. However, given the importance of these parameters we propose to include standardized screening assays for antimicrobial resistance development, anti-biofilm activity, and ability to eradicate persister cells in a second phase of antibiotic lead optimization (**Figure 1**). Moreover, preclinical assessment of antibiotic leads using pharmacokinetic and pharmacodynamic (PK/PD) studies has proven critical to determine the optimal dosing for subsequent animal and clinical studies [13, 15, 16]. Pharmacokinetics describe the distribution of antimicrobial agent concentration over time, while pharmacodynamics define the relationship between these concentrations and the overall antimicrobial activity of the agent. Such studies thus provide information on peak concentrations reached in the blood stream after administration, time range in which the MIC is reached, tissue distribution and clearance period of the agent, thereby improving success rates and accelerating antibiotic drug discovery [15, 16]. Given the importance of PK/PD characteristics in the success rate of antimicrobial agents, it should be considered to include PK/PD screening in an earlier stage of antibiotic drug development (**Figure 1**). Nevertheless, such studies are very expensive and with the limited funding available for antibiotic drug discovery, this would require innovations in financial support of antibiotic drug discovery programs that go beyond the push and pull strategies currently in place [17].

### 2.2. *In vitro* assays predictive for *in vivo* activities of AMPs should include 3D human infection models and biological fluids

From an ethical point of view, it would be preferred to use *in vitro* assays over *in vivo* models to determine the activities of novel antimicrobial agents as SAAP-148 or Ab-Cath. Concurrently, these assays should predict for *in vivo* activities of these agents. Taking cytotoxicity as an example, we observed in **chapters 3, 5 and 6** that cytotoxicity of SAAP-148 and Ab-Cath was most prominent towards human erythrocytes, followed by monolayers of cells, while 3D human epidermal models and 3D human urothelial models were proven to be much more resistant to the cytotoxic

activities of these AMPs. Additionally, cutaneous application of SAAP-148 has previously proven to be safe in an animal model [18]. Together, these data describe the correlation between cytotoxicity of the AMP and complexity of the cell model, with single cells being much more prone to cytotoxicity, while complex 3D models and *ex vivo* or *in vivo* skin are much more resistant. Similarly, Greco et al. reported reduced cytotoxicity of AMPs to monolayers of cells compared to human erythrocytes, and importantly reported that the *in vitro* cytotoxicity was not observed in their *in vivo* model [19]. Therefore, we believe that cell suspensions and monolayers are very

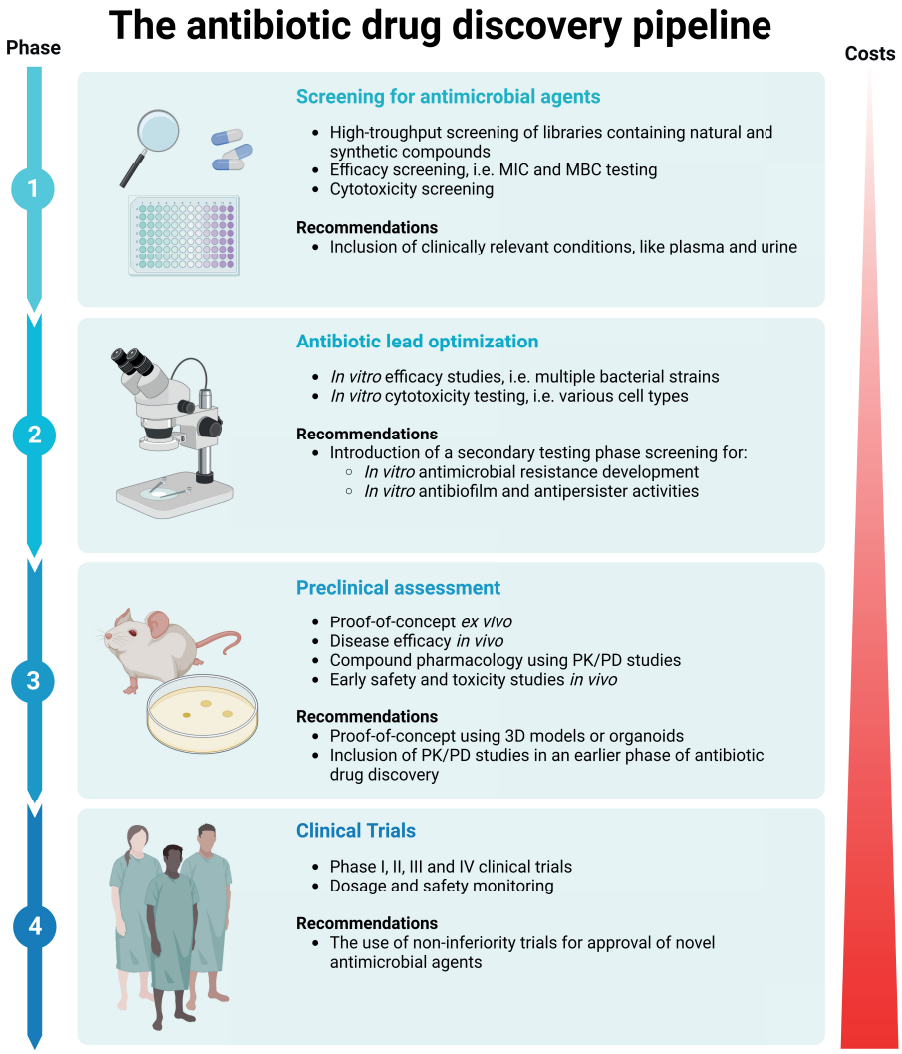



**Figure 1. The antibiotic drug discovery pipeline.** An overview of the four phases of antibiotic drug discovery, including recommendations made to improve discovery of antibiotic leads for successful implementation in the clinic. These four phases are important for final approval by authorities.

useful for quick cytotoxicity screening of a library of novel antibacterial agents, but that the cytotoxic concentrations found in these screenings do not accurately predict for *in vivo* toxicity. Although 3D human epidermal models have been reported to be more permeable compared to human skin [20], they do resemble the human skin in many ways [21]. Based on our data in **chapters 3, 5 and 6**, we propose to include such 3D models and/or organoids during preclinical assessment (**Figure 1**), as it is a more realistic predictor of *in vivo* cytotoxicity compared to using cell suspensions or monolayers and could serve as a proof-of-concept before moving to *in vivo* studies. A similar correlation can be described for the antimicrobial activities of SAAP-148 and Ab-Cath when moving from *in vitro* to *ex vivo* and ultimately to *in vivo*. In **chapter 3, 5 and 6** we observed that planktonic bacteria were most easily eradicated by these AMPs, followed by planktonic bacteria colonizing 3D models, and finally biofilm-residing bacteria. Furthermore, Dijksteel et al. and de Breij et al. demonstrated that *ex vivo* and *in vivo* bacterial infections were much more resistant to SAAP-148 treatment, requiring high SAAP-148 concentrations [18, 22]. Moreover, standard *in vitro* screening assays are often performed in biologically irrelevant conditions. Nevertheless, it is known that antimicrobial activities and also cytotoxic activities of AMPs are reduced in presence of biological fluids like human plasma, urine, eschar extract or proteolytic enzymes [18, 22-28]. The reduced antimicrobial and cytotoxic activities of SAAP-148 in presence of human plasma and urine compared to saline solution were also reported in **chapters 3 and 6**. Depending on the clinical application, inclusion of biological fluids and host cells in standardized testing conditions allows to mimic the clinical infection more closely.

### 2.3. The use of non-inferiority trials for approval of novel antimicrobial agents in an age of AMR

For novel antibiotics or alternatives to reach the clinic, their effectivity has to be confirmed in clinical superiority or non-inferiority trials [29]. Superiority trials are designed such that the antimicrobial agent should be more effective than the standard of care treatment. Surotomycin, pexiganan and omiganan are examples of AMPs that failed to show superiority over conventional antibiotics, but could be promising alternatives due to an improved safety profile or reduced ability to induce bacterial resistance [30, 31]. However, to prove the superior effect of such AMPs in a clinical trial would require the inclusion of patients with bacterial infections resistant to the standard of care treatment [32], which would be extremely costly. Alternatively, the clinical trial would require the comparison to a control antibiotic that is predicted to be ineffective as a consequence of AMR development, which is highly unethical, especially when there is high risk of morbidity or mortality [29]. With the global rise of AMR, it would therefore benefit public health care if noninferiority trials are included with the aim to approve novel antimicrobial agents that are as good

as standard of care antibiotics but have added benefits, such as low risk of AMR development (**Figure 1**). It should be noted that noninferiority trials are associated with a low risk of approval of consistently less effective antibiotics, but this risk can be further mitigated by including antibiotics with the best-estimated treatment effect as comparator [33-35].

## 3. Critical factors in chemical lead optimization of AMPs

### 3.1. Optimization of AMP characteristics is critical for their success in the clinic

In general, AMPs have promising antimicrobial activities, but often suffer from a limited selectivity towards bacteria over mammalian cells as they can be rather cytotoxic [10, 30, 36]. The main focus of AMP lead optimization should therefore be the reduction of cytotoxicity while maintaining antimicrobial activity. At the same time, stability of the AMP is an important characteristic [10, 30, 36], such that the promising *in vitro* activities of the AMP are maintained in *in vivo* models. Multiple chemical modifications have been researched to improve these characteristics of AMPs, including C- and N-terminal end-capping, incorporation of D-amino acids, using retro-inverse structures, changing the length and/or amino acid sequence or conjugation to other molecules [37-42]. Another big hurdle in AMP drug development are the costs related to production of the AMP [10, 30, 36]. Therefore, it is important to focus on truncated and/or short AMP leads, which have increased chances of being used in the clinic. Based on these criteria and the findings in **chapter 2** of this thesis, we would recommend to focus on short AMP leads during AMP development and improve upon their characteristics by C- and N-terminal end-capping to enhance stability and by PEGylation with a short PEG chain to reduce cytotoxicity of the AMP lead.

### 3.2. Immunomodulatory activities of AMPs as additional mechanism to reduce AMR development

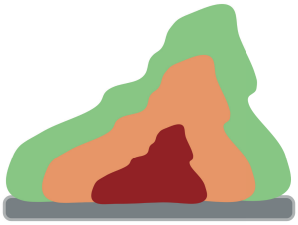
Initially, AMPs were recognized for their direct antimicrobial activities, while some of these peptides also have shown to exhibit immunomodulatory properties, hence their alternative name host defense peptides (HDPs) [43, 44]. This dual activity defines AMPs or HDPs as truly interesting peptides that can eradicate bacteria by their direct activities and/or by their indirect host-directed mechanisms modulating immune cells involved in both innate and adaptive immunity [44-46]. Host-directed therapy for treatment of bacterial infections provides an alternative approach that is i) effective against AMR bacteria, ii) less likely to induce bacterial resistance and iii) has potential to be synergistic or additive to alternative therapies [47]. We have demonstrated in **chapter 2** that PEGylation of SAAP-148 strengthened the intrinsic immunomodulatory properties of this AMP, without affecting its direct antimicrobial

activities. More specifically, PEGylated SAAP-148 was able to skew an anti-inflammatory immune landscape to a pro-inflammatory environment. Such a shift is important to eradicate bacteria that persist as a result of suppressed immune responses, but could also be of importance in context of tumors that locally polarize macrophages to an anti-inflammatory subset to evade the immune system [48, 49]. Therefore, PEGylated AMPs hold promise as dual acting therapy to combat bacterial infections and as novel strategy to initiate tumor regression via redirection of inadequate anti-inflammatory immune responses in the tumor microenvironment.

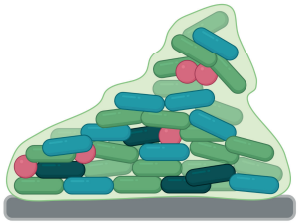
## 4. The importance of using AMPs in combination with other therapeutic agents

### 4.1. Improved activity through additive or synergistic AMP-antibiotic combinations

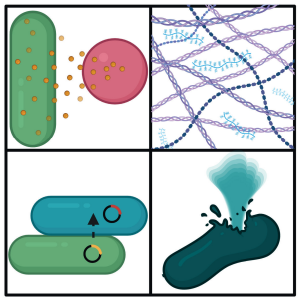
The therapeutic potential of an AMP lead, especially when it has a mode of action different from current antibiotics, could increase when combined with an antibiotic. Synergistic AMP-antibiotic combinations achieve the same antimicrobial activity at a lower AMP concentration, so this approach requires a lower individual drug dosage and thus is a more safe approach [50]. Moreover, synergistic AMP-antibiotic combinations are less likely to induce AMR [51, 52]. The idea of combination therapy is not new and has been exploited for several other infectious diseases over the past decade, including tuberculosis [53, 54]. Initially, tuberculosis patients were treated with streptomycin alone, but treatment was extended with an additional drug, para-aminosalicylic acid, to overcome antimicrobial resistance due to resistance to streptomycin monotherapy, and nowadays, a four-drug regimen is employed for treatment of tuberculosis [55]. Considering that combination therapies usually build upon the standard of care treatment, it would be important to screen novel antimicrobial agents for synergistic effects with antibiotics currently used in the clinic, which include fluorquinolones, macrolides, tetracyclines and beta-lactams among others [56]. Previously, synergy has been described for SAAP-148 when used in combination with the classical antibiotics teicoplanin [57] and demeclocycline [58]. Moreover, synergy was described for SAAP-148 in combination with the novel antibiotic halicin in **chapter 3** of this thesis. This study confirms the benefits of using the SAAP-148-halicin combination, as lower, non-cytotoxic SAAP-148 concentrations could be used to reach the same antimicrobial activities. Importantly, this study proved that the favorable interactions between SAAP-148 and halicin were also observed in 3D models that mimic the urothelial tract and the skin epidermis, indicating that synergistic effects observed *in vitro* do hold in 3D models mimicking clinical bacterial infections.



**Biofilm subpopulations**
- Antibiotic-susceptible cells (green zone)
- Antibiotic-tolerant cells (orange zone)
- Persister cells (red zone)
- EPS-producing cells (throughout)



**Biofilm resistance mechanisms**
- Multiple species involved
- Incubator for gene exchange
- Interplay between subpopulations
- Multiple microenvironments
- Limited diffusion by antibiotics or host cells



**Antibiofilm targets**
- Quorum sensing processes
- Extracellular polymeric substances (EPS)
- EPS synthesis, EPS adhesins or lectins
- Conjugation-associated targets
- Bacterial metabolism
- Bacterial cell membrane

**Figure 2. Biofilm subpopulations, resistance mechanisms and antibiofilm targets.** Schematic overview of the different subpopulations of the bacterial biofilm. EPS-producing cells are found throughout the biofilm, while antibiotic-susceptible cells, antibiotic-tolerant cells and persisters are observed moving from the outer surface to the inside of the biofilm. The resistance of bacterial biofilms to antibiotics depends partly on the presence of these subpopulations, but also involves other mechanisms, such as multi-species company, exchange of genes and production of extracellular polymeric substances. Therefore, bacterial biofilms can be targeted by inhibiting multiple processes, like quorum sensing, EPS synthesis or bacterial conjugation. Figure is based on Bisht and Wakeman [59].

### 4.2. Synergetic AMP combinations to eradicate bacterial biofilms

Bacterial biofilms are present in approximately 80% of chronic and recurrent bacterial infections [4], therefore it is of most importance to develop an effective antibiofilm therapy. The biofilm comprises multiple subpopulations, including antibiotic-susceptible cells, antibiotic-tolerant cells, persister cells and EPS producing cells [59], that all contribute to resistance of the biofilm against therapy (**Figure 2**). AMP-antibiotic combinations have been shown to synergize against bacterial biofilms [57, 60], as was also demonstrated in **chapter 3** of this thesis for SAAP-148 and halicin. To further enhance the antibiofilm activities of AMPs, these peptides could be combined

with other agents that target bacterial biofilms, such as anti-quorum sensing agents, matrix degrading enzymes and bacterial conjugation inhibitors (**Figure 2**). The process of quorum sensing, i.e. the ability of bacteria to communicate and regulate gene expression via signaling molecules, plays a vital role in biofilm development [61]. Importantly, AMPs have been combined successfully with quorum sensing inhibitors, such as RNA III-inhibiting peptide and a derivative thereof, to improve antibiofilm activities [61-63]. Another important biofilm target comprises the bacterial-produced extracellular polymeric substances (EPS) that not only shape the biofilm matrix as a layer of protection, but also promote bacterial adherence to surfaces, cell-cell adhesion and aggregation [61]. Matrix degrading enzymes and chelating agents interfering with iron-dependent metabolic processes have been shown to synergize with AMPs and more efficiently eradicated bacterial biofilms [64-66]. Alternatively, bacterial biofilms could be targeted by inhibition of EPS synthesis or by binding to EPS adhesins or lectins to prevent initial adhesion of bacteria to cellular surfaces [59, 61, 65, 67]. Moreover, bacterial conjugation inhibitors, such as unsaturated fatty acids and derivatives thereof, can prevent antimicrobial resistance development in the biofilm by blocking horizontal gene exchange [68]. A burdensome subpopulation within biofilm communities comprises persister cells, which are difficult to treat due to their metabolically inactive state. This subpopulation could be specifically targeted using agents that stimulate persister cell metabolism or by agents that disrupt the bacterial cell membrane instead [59]. The combined administration of two diverse agents, as is the case with AMPs and antibiotics or antibiofilm agents, can face difficulties related to differences in PK/PD properties. Conjugation of the two agents could be considered as a strategy to overcome this limitation [69, 70].

## 5. The future of AMP-loaded DDSs

### 5.1. From application to design of an AMP-loaded DDS

The first drug DDS allowing controlled-release of a drug was developed in the 1950's, and since then drug delivery technology has advanced significantly [71]. Nowadays, multiple drug delivery approaches are available and used for AMPs [72], including nanosized lipid and polymeric DDSs and coatings that are described in **chapter 4** of this thesis. These AMP DDSs offer several advantages for application of AMPs, including improved stability and bioavailability [73-75], reduced cytotoxicity [76, 77] and improved biofilm penetration and intracellular retention [77-79]. Moreover, the properties of these AMP-loaded DDSs can be tuned to specific clinical applications, that require different operational conditions. Previously, Liu et al. concluded that the ideal diameter of a DDS targeting bacterial biofilms ranged between 5 and 100-200 nm based on the channel dimensions within the biofilm, but should not exceed 500 nm to prevent recognition by the immune system leading to quick clearance from the

circulation [80]. Moreover, functionalization of DDSs with hydrophilic polymers, like PEG, can allow stealth transport of the DDS through the bloodstream and improve their biocompatibility [80]. Importantly, based on current information available on AMP-loaded DDSs we made recommendations for most promising DDSs to protect and deliver AMPs to combat hard-to-treat infections (**chapter 4**). For bloodstream and deep-seated infections soft nanoparticles and fusogenic liposomes could be advantageous, because they protect AMPs from degradation, enhance AMP circulation time and allow AMP transport through tissues. In contrast, for pulmonary and intracellular infections cationic solid nanoparticles would be preferred, that favor penetration in mucus and biofilms, and allow internalization into epithelial cells. On the other hand, catheter-related and implant-associated infections could be best prevented using layer-by-layer AMP coatings, that prevent introduction of bacterial pathogens, eliminate bacterial growth on the material surface and reduce bacterial growth in the surrounding tissue. For complex wound infections anionic hydrophilic nanoparticles might be beneficial, that protect AMPs from degradation, but subsequently improve biofilm penetration properties and provide sustained AMP release over time. Together, the clinical application and aimed characteristics of an AMP-loaded DDSs should be absolutely clear from the start of a research project, in order to accurately design an AMP-loaded DDS for successful implementation in the clinic.

**5.2. Challenges for translation of AMP-loaded DDSs to the clinic**

Regardless of the advantages of DDSs for AMP delivery, significant challenges have to be overcome to produce an effective pharmacological product for clinical application in infectious diseases. One of the challenges is related to the lack of standardized testing of AMP DDSs, complementary to the discussion in **paragraphs 2.1 and 2.2**. Likewise, these DDSs are mostly tested using simple MIC assays, but are inadequately evaluated in more complex situations involving biofilms, persister cells, AMR strains or relevant biological conditions, i.e. plasma or urine (**chapter 4**). We addressed part of this *in vitro* knowledge gap by thoroughly evaluating SAAP-148 and Ab-Cath nanogel formulations against planktonic, biofilm-residing and skin-associated AMR bacterial strains, as well as evaluating cytotoxicity against erythrocytes, human skin fibroblast and keratinocyte monolayers, and in a 3D human epidermal model (**chapters 5 and 6**). Besides the lack of proper evaluation of a single AMP-loaded DDS, there are limited studies comparing multiple DDSs for one specific application. Introduction of such studies could validate our recommendations for the best-of-choice DDS for the envisioned clinical application, which were presented in **paragraph 4.1**. Moreover, *in vivo* experiments are crucial to determine the true efficacy and safety, and PK/PD properties of AMPs loaded in DDSs. Considering the increasing lack of effective treatment of antimicrobial resistant

infections, it is key to evaluate AMP-loaded DDSs *in vivo* as quickly as possible. Another challenge to produce an AMP DSSs for clinical application in infectious diseases is related to the lack of shelf-stable DDSs. Despite the promising activities of lipid and polymeric DDSs loaded with AMPs, the development of a shelf-stable pharmaceutical formulation is crucial for successful implementation of these DDSs in the clinic. Lyophilization of the pharmacological product is a common strategy to allow long-term storage and easy distribution, improve product shelf-life and enhance formulation stability [81]. Importantly, for the first time we developed a successful lyophilization method for AMP-loaded hyaluronic acid-based nanogels using snake cathelicidin Ab-Cath (**chapter 5**). This method was successfully employed for SAAP-148-loaded hyaluronic acid-based nanogels (**chapter 6**), emphasizing the general applicability of the lyophilization method.

### 5.3. AMP-loaded DDSs targeting bacterial biofilms

A range of DDSs has shown potential to improve biofilm penetration, including liposomes and polymeric nanoparticles [82]. Nevertheless, we did not observe improved antibiofilm activities for both Ab-Cath- and SAAP-148-loaded hyaluronic acid-based nanogels compared to the peptides in solution (**chapters 5 and 6**). Functionalization of these DDSs might be a subsequent approach to enhance their biofilm penetration and antibiofilm activities. For example, monoclonal antibodies targeting surface proteins of *Staphylococcus aureus* have been described by de Vor et al. to specifically recognize *S. aureus* biofilms [83], and could be used to coat surfaces of DDSs to target bacterial biofilms and improve localization. Alternatively, Sharma et al. functionalized IDR-1018-loaded PLGA nanoparticles with the biofilm disrupting agent N-acetyl cysteine and demonstrated improved antibiofilm activities of their DDS compared to the nonfunctionalized counterpart [84]. Moreover, the synergistic strategies described in **paragraph 4** are also used for AMP-loaded DDSs. For instance, Faya et al. demonstrated an improved antimicrobial activity for vancomycin-loaded liposomes decorated with AMPs against *S. aureus* compared to single-loaded DDSs [78]. Alternatively, smart DDSs are being developed that are responsive to specific stimuli, such as light, temperature, pH and/or specific enzymes [82, 85]. Several studies have combined the above mentioned strategies to develop novel DDSs with antibiofilm activities. For instance, Hu et al. developed photothermal nanoparticles coated with polydopamine, that kill bacteria by producing heat locally after near-infrared light irradiation [86]. In addition, their DDS efficiently targeted methicillin-resistant *S. aureus* due to additional surface functionalization with vancomycin. Moreover, Meeker et al. loaded an antibiotic in photothermal polydopamine-coated gold nanoparticles, and demonstrated synergistic photothermal- and antibiotic-mediated killing of *S. aureus* and *Pseudomonas aeruginosa* [87, 88]. In addition, these nanoparticles showed

good antibiofilm properties after functionalization with antibodies targeting specific lipoproteins, which are highly produced in biofilms. Likewise, gold nanocages were targeted to bacteria by coating with macrophage membranes containing bacterial recognizing receptors [89]. Bacteria-specific targeting could also be achieved by functionalization of the DDS with aptamers or lectins [90-93]. In addition, Liu et al. described pH adaptive micellar nanocarriers that change charge from anionic to cationic in the low pH environment of bacterial biofilms, improving the penetration and retention in the biofilm. Moreover, this system was complemented by using light-activatable antibacterial agents, resulting in a superior system compared to its nonresponsive counterpart [94]. As mentioned previously, DDSs could improve the selectivity index of the loaded antibacterial agent. Importantly, we observed improved selectivity indices for both Ab-Cath and SAAP-148 upon formulation in hyaluronic acid-based nanogels (**chapters 5 and 6**). Some of the above mentioned strategies allow to selectively target the site of infection followed by on-demand release, and thus have promise to further enhance the selectivity index of AMPs loaded in DDSs.

**6. Conclusions and future directions**

This thesis presents three strategies to improve lead AMP SAAP-148, namely i) chemical lead-optimization using PEGylation, ii) combination therapy with novel antibiotic halicin and iii) development of hyaluronic acid-based nanogels for delivery of SAAP-148. The latter strategy was also employed for snake cathelicidin Ab-Cath. The most important findings of this thesis are summarized below. Firstly, PEGylation of SAAP-148 resulted in reduced cytotoxicity towards erythrocytes and remarkably improved the ability of the peptide to modulate the immune system to a more pro-inflammatory subset, i.e. enhancing neutrophil migration, (re)directing monocyte-macrophage differentiation towards pro-inflammatory macrophages (type 2) and promoting maturation of dendritic cells during the monocyte-dendritic cell differentiation process. Secondly, synergy was discovered between SAAP-148 and novel antibiotic halicin against AMR *S. aureus* and *E. coli*. Importantly, the favorable interactions were maintained in biofilms and clinically relevant 3D human infection models. Thirdly, formulation of SAAP-148 and Ab-Cath in hyaluronic acid-based nanogels maintained their antimicrobial activities against AMR bacterial strains and reduced their cytotoxic activities, thus improving the selectivity indices of these peptides. In conclusion, the results in the present thesis contribute to the development of SAAP-148 and Ab-Cath as therapeutics to combat bacterial infections. Finally, we presented future directions for further development of AMPs in this chapter. In general, the success rate of antibiotic drug discovery should be improved by i) incorporation of clinically relevant conditions in early *in vitro* screenings, ii) using 3D models as proof-of-concept for *in vivo* studies and iii) utilizing non-inferiority clinical trials for approval of novel antibiotics. Moreover, to further improve upon the

promising activities of AMPs, these peptides should be used in combination with antibiotics or other antimicrobial agents. Additionally, combinations of AMPs and antibiotics or antimicrobial agents further reduce AMR development and allow for development of an effective antibiofilm therapy. Ultimately, AMPs (or combinations) should be incorporated in DDSs for clinical application. These systems allow for protection of the AMP, improve PK/PD properties and can be functionalized to target bacterial biofilm infections. Together, these directions might be the way forward to introduce these AMPs to the clinic.

## References


1. Iskandar K, Murugaiyan J, Hammoudi Halat D, Hage SE, Chibabhai V, Adukkadukkam S, et al. Antibiotic Discovery and Resistance: The Chase and the Race. Antibiotics (Basel). 2022;11(2).
2. Penesyan A, Gillings M, Paulsen IT. Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. Molecules. 2015;20(4):5286-98.
3. Lewis K. Multidrug tolerance of biofilms and persister cells. Curr Top Microbiol Immunol. 2008;322:107-31.
4. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019;8:76.
5. Cook MA, Wright GD. The past, present, and future of antibiotics. Sci Transl Med. 2022;14(657):eabo7793.
6. Li W, Separovic F, O'Brien-Simpson NM, Wade JD. Chemically modified and conjugated antimicrobial peptides against superbugs. Chem Soc Rev. 2021;50(8):4932-73.
7. Magana M, Pushpanathan M, Santos AL, Leanse L, Fernandez M, Ioannidis A, et al. The value of antimicrobial peptides in the age of resistance. Lancet Infect Dis. 2020;20(9):e216-e30.
8. Nguyen LT, Haney EF, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. Trends Biotechnol. 2011;29(9):464-72.
9. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(6870):389-95.
10. Barreto-Santamaria A, Patarroyo ME, Curtidor H. Designing and optimizing new antimicrobial peptides: all targets are not the same. Crit Rev Cl Lab Sci. 2019;56(6):351-73.
11. Kang SJ, Park SJ, Mishig-Ochir T, Lee BJ. Antimicrobial peptides: therapeutic potentials. Expert Rev Anti-Infe. 2014;12(12):1477-86.
12. Ulvatne H. Antimicrobial peptides - Potential use in skin infections. Am J Clin Dermatol. 2003;4(9):591-5.
13. Miethke M, Pieroni M, Weber T, Bronstrup M, Hammann P, Halby L, et al. Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem. 2021;5(10):726-49.
14. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163-75.
15. Rodriguez-Gascon A, Solinis MA, Isla A. The Role of PK/PD Analysis in the Development and Evaluation of Antimicrobials. Pharmaceutics. 2021;13(6).
16. Velkov T, Bergen PJ, Lora-Tamayo J, Landersdorfer CB, Li J. PK/PD models in antibacterial development. Curr Opin Microbiol. 2013;16(5):573-9.
17. Dutescu IA, Hillier SA. Encouraging the Development of New Antibiotics: Are Financial Incentives the Right Way Forward? A Systematic Review and Case Study. Infect Drug Resist. 2021;14:415-34.
18. de Breij A, Riool M, Cordfunke RA, Malanovic N, de Boer L, Koning RI, et al. The antimicrobial peptide SAAP-148 combats drug-resistant bacteria and biofilms. Sci Transl Med. 2018;10(423).
19. Greco I, Molchanova N, Holmedal E, Jenssen H, Hummel BD, Watts JL, et al. Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. Sci Rep-Uk. 2020;10(1).
20. Thakoersing VS, Gooris GS, Mulder A, Rietveld M, El Ghalbzouri A, Bouwstra JA. Unraveling barrier properties of three different in-house human skin equivalents. Tissue Eng Part C Methods. 2012;18(1):1-11.
21. Reijnders CM, van Lier A, Roffel S, Kramer D, Scheper RJ, Gibbs S. Development of a Full-Thickness Human Skin Equivalent In Vitro Model Derived from TERT-Immortalized Keratinocytes and Fibroblasts. Tissue Eng Part A. 2015;21(17-18):2448-59.
22. Dijksteel GS, Ulrich MMW, Nibbering PH, Cordfunke RA, Drijfhout JW, Middelkoop E, et al. The functional stability, bioactivity and safety profile of synthetic antimicrobial peptide SAAP-148. Journal of Microbiology and Antimicrobials. 2020;12(2):70-80.
23. Burian A, Wagner C, Stanek J, Manafi M, Bohmdorfer M, Jager W, et al. Plasma protein binding may reduce antimicrobial activity by preventing intra-bacterial uptake of antibiotics, for example clindamycin. J Antimicrob Chemoth. 2011;66(1):134-7.
24. McCrudden MTC, McLean DTF, Zhou M, Shaw J, Linden GJ, Irwin CR, et al. The Host Defence Peptide LL-37 is Susceptible to Proteolytic Degradation by Wound Fluid Isolated from Foot Ulcers of Diabetic Patients. Int J Pept Res Ther. 2014;20(4):457-64.

25. Moncla BJ, Pryke K, Rohan LC, Graebing PW. Degradation of naturally occurring and engineered antimicrobial peptides by proteases. Adv Biosci Biotechnol. 2011;2(6):404-8.
26. Pappen FG, Qian W, Aleksejuniene J, Leonardo Rde T, Leonardo MR, Haapasalo M. Inhibition of sodium hypochlorite antimicrobial activity in the presence of bovine serum albumin. J Endod. 2010;36(2):268-71.
27. Quintana RM, Jardine AP, Montagner F, Fatturi Parolo CC, Morgental RD, Poli Kopper PM. Effect of human, dentin, albumin and lipopolysaccharide on the antibacteerial activity of endodontic activity of endodontic irrigants. J Conserv Dent. 2017;20(5):341-5.
28. Starr CG, Wimley WC. Antimicrobial peptides are degraded by the cytosolic proteases of human erythrocytes. Biochim Biophys Acta Biomembr. 2017;1859(12):2319-26.
29. Rex JH, Fernandez Lynch H, Cohen IG, Darrow JJ, Outterson K. Designing development programs for non-traditional antibacterial agents. Nat Commun. 2019;10(1):3416.
30. Dijksteel GS, Ulrich MMW, Middelkoop E, Boekema B. Review: Lessons Learned From Clinical Trials Using Antimicrobial Peptides (AMPs). Front Microbiol. 2021;12:616979.
31. Gordon YJ, Romanowski EG, McDermott AM. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr Eye Res. 2005;30(7):505-15.
32. Lanini S, Ioannidis JPA, Vairo F, Pletschette M, Portella G, Di Bari V, et al. Non-inferiority versus superiority trial design for new antibiotics in an era of high antimicrobial resistance: the case for post-marketing, adaptive randomised controlled trials. Lancet Infect Dis. 2019;19(12):e444-e51.
33. Bai AD, Komorowski AS, Lo CKL, Tandon P, Li XNX, Mokashi V, et al. Novel Antibiotics May Be Noninferior but Are They Becoming Less Effective?: a Systematic Review. Antimicrob Agents Ch. 2020;64(11).
34. Odem-Davis K, Fleming TR. A simulation study evaluating bio-creep risk in serial non-inferiority clinical trials for preservation of effect. Stat Biopharm Res. 2015;7(1):12-24.
35. Rex JH, Talbot GH, Goldberger MJ, Eisenstein BI, Echols RM, Tomayko JF, et al. Progress in the Fight Against Multidrug-Resistant Bacteria 2005-2016: Modern Noninferiority Trial Designs Enable Antibiotic Development in Advance of Epidemic Bacterial Resistance. Clin Infect Dis. 2017;65(1):141-6.
36. Marr AK, Gooderham WJ, Hancock REW. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol. 2006;6(5):468-72.
37. Kindrachuk J, Scruten E, Attah-Poku S, Bell K, Potter A, Babiuk LA, et al. Stability, toxicity, and biological activity of host defense peptide BMAP28 and its inversed and retro-inversed isomers. Biopolymers. 2011;96(1):14-24.
38. Morris CJ, Beck K, Fox MA, Ulaeto D, Clark GC, Gumbleton M. Pegylation of Antimicrobial Peptides Maintains the Active Peptide Conformation, Model Membrane Interactions, and Antimicrobial Activity while Improving Lung Tissue Biocompatibility following Airway Delivery. Antimicrob Agents Ch. 2012;56(6):3298-308.
39. Nguyen LT, Chau JK, Perry NA, de Boer L, Zaat SA, Vogel HJ. Serum stabilities of short tryptophan- and arginine-rich antimicrobial peptide analogs. PLoS One. 2010;5(9).
40. Ramesh S, Govender T, Kruger HG, de la Torre BG, Albericio F. Short AntiMicrobial Peptides (SAMPs) as a class of extraordinary promising therapeutic agents. Journal of Peptide Science. 2016;22(7):438-51.
41. Zeiders SM, Chmielewski J. Antibiotic-cell-penetrating peptide conjugates targeting challenging drug-resistant and intracellular pathogenic bacteria. Chem Biol Drug Des. 2021;98(5):762-78.
42. Zhao Y, Zhang M, Qiu S, Wang J, Peng J, Zhao P, et al. Antimicrobial activity and stability of the D-amino acid substituted derivatives of antimicrobial peptide polybia-MPI. AMB Express. 2016;6(1):122.
43. Haney EF, Mansour SC, Hancock RE. Antimicrobial Peptides: An Introduction. Methods Mol Biol. 2017;1548:3-22.
44. Mookherjee N, Anderson MA, Haagsman HP, Davidson DJ. Antimicrobial host defence peptides: functions and clinical potential. Nat Rev Drug Discov. 2020;19(5):311-32.
45. Bowdish DME, Davidson DJ, Scott MG, Hancock REW. Immunomodulatory activities of small host defense peptides. Antimicrob Agents Ch. 2005;49(5):1727-32.
46. Hilchie AL, Wuerth K, Hancock RE. Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. Nat Chem Biol. 2013;9(12):761-8.
47. Kilinc G, Saris A, Ottenhoff THM, Haks MC. Host-directed therapy to combat mycobacterial infections. Immunol Rev. 2021;301(1):62-83.

48. Chen YB, Song YC, Du W, Gong LL, Chang HC, Zou ZZ. Tumor-associated macrophages: an accomplice in solid tumor progression. J Biomed Sci. 2019;26(1).
49. Etzerodt A, TsaLkitzi K, Maniecki M, Damsky W, Delfini M, Baudoin E, et al. Specific targeting of CD163(+) TAMs mobilizes inflammatory monocytes and promotes T cell-mediated tumor regression. J Exp Med. 2019;216(10):2394-411.
50. Yu G, Baeder DY, Regoes RR, Rolff J. Combination Effects of Antimicrobial Peptides. Antimicrob Agents Chemother. 2016;60(3):1717-24.
51. Lewies A, Du Plessis LH, Wentzel JF. Antimicrobial Peptides: the Achilles' Heel of Antibiotic Resistance? Probiotics Antimicrob Proteins. 2019;11(2):370-81.
52. Xu XJ, Xu L, Yuan GJ, Wang YM, Qu YQ, Zhou MJ. Synergistic combination of two antimicrobial agents closing each other's mutant selection windows to prevent antimicrobial resistance. Sci Rep-Uk. 2018;8.
53. Coates ARM, Hu Y, Holt J, Yeh P. Antibiotic combination therapy against resistant bacterial infections: synergy, rejuvenation and resistance reduction. Expert Rev Anti Infect Ther. 2020;18(1):5-15.
54. Fischbach MA. Combination therapies for combating antimicrobial resistance. Curr Opin Microbiol. 2011;14(5):519-23.
55. Kerantzas CA, Jacobs WR, Jr. Origins of Combination Therapy for Tuberculosis: Lessons for Future Antimicrobial Development and Application. mBio. 2017;8(2).
56. van den Broek AK, van Hest RM, Lettinga KD, Jimmink A, Lauw FN, Visser CE, et al. The appropriateness of antimicrobial use in the outpatient clinics of three hospitals in the Netherlands. Antimicrob Resist Infect Control. 2020;9(1):40.
57. Koppen BC, Mulder PPG, de Boer L, Riool M, Drijfhout JW, Zaat SAJ. Synergistic microbicidal effect of cationic antimicrobial peptides and teicoplanin against planktonic and biofilm-encased Staphylococcus aureus. Int J Antimicrob Ag. 2019;53(2):143-51.
58. Li SJ, She PF, Zhou LY, Zeng XH, Xu LL, Liu YQ, et al. High-Throughput Identification of Antibacterials Against Pseudomonas aeruginosa. Frontiers in Microbiology. 2020;11.
59. Bisht K, Wakeman CA. Discovery and Therapeutic Targeting of Differentiated Biofilm Subpopulations. Frontiers in Microbiology. 2019;10.
60. Duong L, Gross SP, Siryaporn A. Developing Antimicrobial Synergy With AMPs. Front Med Technol. 2021;3:640981.
61. Srinivasan R, Santhakumari S, Poonguzhali P, Geetha M, Dyavaiah M, Xiangmin L. Bacterial Biofilm Inhibition: A Focused Review on Recent Therapeutic Strategies for Combating the Biofilm Mediated Infections. Front Microbiol. 2021;12:676458.
62. Cirioni O, Mocchegiani F, Cacciatore I, Vecchiet J, Silvestri C, Baldassarre L, et al. Quorum sensing inhibitor FS3-coated vascular graft enhances daptomycin efficacy in a rat model of staphylococcal infection. Peptides. 2013;40:77-81.
63. Balaban N, Gov Y, Giacometti A, Cirioni O, Ghiselli R, Mocchegiani F, et al. A chimeric peptide composed of a dermaseptin derivative and an RNA III-inhibiting peptide prevents graft-associated infections by antibiotic-resistant staphylococci. Antimicrob Agents Chemother. 2004;48(7):2544-50.
64. Gawande PV, Leung KP, Madhyastha S. Antibiofilm and antimicrobial efficacy of DispersinB(R)-KSL-W peptide-based wound gel against chronic wound infection associated bacteria. Curr Microbiol. 2014;68(5):635-41.
65. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017;15(12):740-55.
66. Grassi L, Maisetta G, Maccari G, Esin S, Batoni G. Analogs of the Frog-skin Antimicrobial Peptide Temporin 1Tb Exhibit a Wider Spectrum of Activity and a Stronger Antibiofilm Potential as Compared to the Parental Peptide. Front Chem. 2017;5.
67. Lewis AL, Kohler JJ, Aebi M. Microbial Lectins: Hemagglutinins, Adhesins, and Toxins. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, et al., editors. Essentials of Glycobiology. 4th ed. Cold Spring Harbor (NY)2022. p. 505-16.
68. Cabezon E, de la Cruz F, Arechaga I. Conjugation Inhibitors and Their Potential Use to Prevent Dissemination of Antibiotic Resistance Genes in Bacteria. Frontiers in Microbiology. 2017;8.
69. Cal PM, Matos MJ, Bernardes GJ. Trends in therapeutic drug conjugates for bacterial diseases: a patent review. Expert Opin Ther Pat. 2017;27(2):179-89.
70. Sheard DE, O'Brien-Simpson NM, Wade JD, Separovic F. Combating bacterial resistance by combination of antibiotics with antimicrobial peptides. Pure Appl Chem. 2019;91(2):199-209.

71. Park K. Controlled drug delivery systems: past forward and future back. J Control Release. 2014;190:3-8.
72. Jampilek J, Kralova K. Advances in Nanostructures for Antimicrobial Therapy. Materials (Basel). 2022;15(7).
73. Carmona-Ribeiro AM, Araujo PM. Antimicrobial Polymer-Based Assemblies: A Review. Int J Mol Sci. 2021;22(11).
74. Mansson R, Frenning G, Malmsten M. Factors Affecting Enzymatic Degradation of Microgel-Bound Peptides. Biomacromolecules. 2013;14(7):2317-25.
75. Thapa RK, Diep DB, Tonnesen HH. Nanomedicine-based antimicrobial peptide delivery for bacterial infections: recent advances and future prospects. J Pharm Invest. 2021;51(4):377-98.
76. Klodzinska SN, Pletzer D, Rahanjam N, Rades T, Hancock REW, Nielsen HM. Hyaluronic acid-based nanogels improve in vivo compatibility of the anti-biofilm peptide DJK-5. Nanomedicine. 2019;20:102022.
77. Ron-Doitch S, Sawodny B, Kuhbacher A, David MMN, Samanta A, Phopase J, et al. Reduced cytotoxicity and enhanced bioactivity of cationic antimicrobial peptides liposomes in cell cultures and 3D epidermis model against HSV. J Control Release. 2016;229:163-71.
78. Faya M, Hazzah HA, Omolo CA, Agrawal N, Maji R, Walvekar P, et al. Novel formulation of antimicrobial peptides enhances antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA). Amino Acids. 2020;52(10):1439-57.
79. Menina S, Eisenbeis J, Kamal MAM, Koch M, Bischoff M, Gordon S, et al. Bioinspired Liposomes for Oral Delivery of Colistin to Combat Intracellular Infections by Salmonella enterica. Adv Healthc Mater. 2019;8(17):e1900564.
80. Liu Y, Shi L, Su L, van der Mei HC, Jutte PC, Ren Y, et al. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev. 2019;48(2):428-46.
81. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process and storage considerations. Adv Drug Deliv Rev. 2006;58(15):1688-713.
82. Sahli C, Moya SE, Lomas JS, Gravier-Pelletier C, Briandet R, Hemadi M. Recent advances in nanotechnology for eradicating bacterial biofilm. Theranostics. 2022;12(5):2383-405.
83. de Vor L, van Dijk B, van Kessel K, Kavanaugh JS, de Haas C, Aerts PC, et al. Human monoclonal antibodies against Staphylococcus aureus surface antigens recognize in vitro and in vivo biofilm. Elife. 2022;11.
84. Sharma A, Vaghasiya K, Gupta P, Singh AK, Gupta UD, Verma RK. Dynamic mucus penetrating microspheres for efficient pulmonary delivery and enhanced efficacy of host defence peptide (HDP) in experimental tuberculosis. J Control Release. 2020;324:17-33.
85. Wang C, Hong T, Cui P, Wang J, Xia J. Antimicrobial peptides towards clinical application: Delivery and formulation. Adv Drug Deliv Rev. 2021;175:113818.
86. Hu D, Zou L, Li B, Hu M, Ye W, Ji J. Photothermal Killing of Methicillin-Resistant Staphylococcus aureus by Bacteria-Targeted Polydopamine Nanoparticles with Nano-Localized Hyperpyrexia. ACS Biomater Sci Eng. 2019;5(10):5169-79.
87. Meeker DG, Jenkins SV, Miller EK, Beenken KE, Loughran AJ, Powless A, et al. Synergistic Photothermal and Antibiotic Killing of Biofilm-Associated Staphylococcus aureus Using Targeted Antibiotic-Loaded Gold Nanoconstructs. ACS Infect Dis. 2016;2(4):241-50.
88. Meeker DG, Wang T, Harrington WN, Zharov VP, Johnson SA, Jenkins SV, et al. Versatility of targeted antibiotic-loaded gold nanoconstructs for the treatment of biofilm-associated bacterial infections. Int J Hyperthermia. 2018;34(2):209-19.
89. Wang C, Wang Y, Zhang L, Miron RJ, Liang J, Shi M, et al. Pretreated Macrophage-Membrane-Coated Gold Nanocages for Precise Drug Delivery for Treatment of Bacterial Infections. Adv Mater. 2018;30(46):e1804023.
90. Chen J, Zhang C, Liu QF, Shao XY, Feng CC, Shen YH, et al. Solanum tuberosum lectin-conjugated PLGA nanoparticles for nose-to-brain delivery: in vivo and in vitro evaluations. J Drug Target. 2012;20(2):174-84.
91. Umamaheshwari RB, Jain NK. Receptor mediated targeting of lectin conjugated gliadin nanoparticles in the treatment of Helicobacter pylori. J Drug Target. 2003;11(7):415-24.
92. Yeom JH, Lee B, Kim D, Lee JK, Kim S, Bae J, et al. Gold nanoparticle-DNA aptamer conjugate-assisted delivery of antimicrobial peptide effectively eliminates intracellular Salmonella enterica serovar Typhimurium. Biomaterials. 2016;104:43-51.

93. Lee B, Park J, Ryu M, Kim S, Joo M, Yeom JH, et al. Antimicrobial peptide-loaded gold nanoparticle-DNA aptamer conjugates as highly effective antibacterial therapeutics against Vibrio vulnificus. Sci Rep-Uk. 2017;7.
94. Liu Y, van der Mei HC, Zhao BR, Zhai Y, Cheng TJ, Li YF, et al. Eradication of Multidrug-Resistant Staphylococcal Infections by Light-Activatable Micellar Nanocarriers in a Murine Model. Adv Funct Mater. 2017;27(44).