Journal of Translational Medicine

REVIEW

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Antimicrobial peptide biological activity, delivery systems and clinical translation status and challenges

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Abstract

Antibiotic resistance is currently one of the most significant threats to global public health and safety. And studies have found that over the next 25 years, 39 million people will die directly and 169 million indirectly due to antibiotic-resistant diseases. Consequently, the development of new types of antimicrobial drugs is urgently needed. Antimicrobial peptides (AMPs) constitute an essential component of the innate immune response in all organisms. They exhibit a distinctive mechanism of action that endows them with a broad spectrum of biological activities, including antimicrobial, antibiofilm, antiviral, and anti-inflammatory effects. However, AMPs also present certain limitations, such as cytotoxicity, susceptibility to protein hydrolysis, and poor pharmacokinetic properties, which have impeded their clinical application. The development of delivery systems can address these challenges by modifying AMP delivery and enabling precise, controlled release at the site of infection or disease. This review offers a comprehensive analysis of the mechanisms of action and biological advantages of AMPs. and systematically evaluate how emerging drug delivery systems, such as nanoparticles and hydrogels, enhance the stability and bioavailability of AMPs, discussing both their strengths and limitations. Moreover, unlike previous reviews, this review highlight the most recent clinically approved AMP-based drugs and those currently in development, emphasizing the key challenges in translating these drugs into clinical practice. With these perspectives, it is hoped that this review will provide some insights into overcoming translational barriers and advancing AMPs drugs into clinical practice.

Keywords Antimicrobial peptides, Biological activity, Clinical translation

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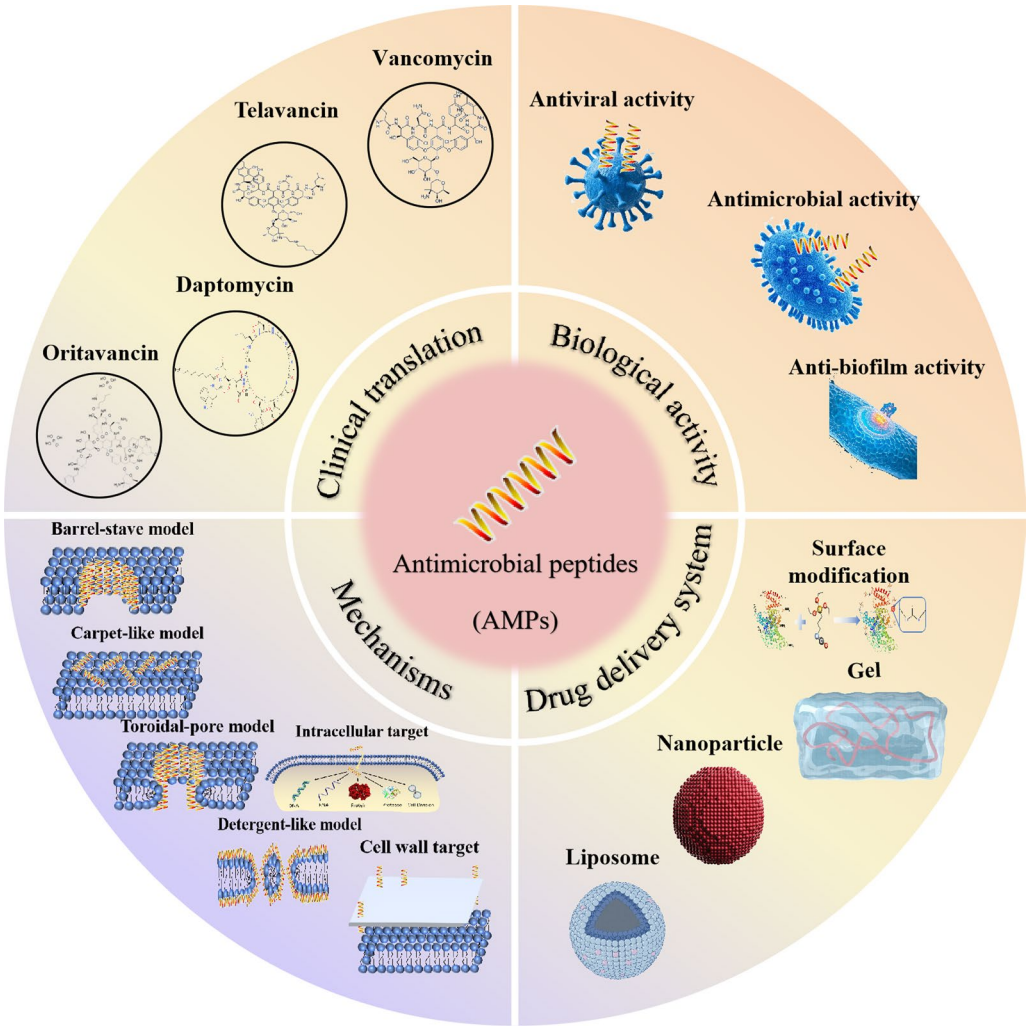
Graphical Abstract



Introduction

The global rise in antibiotic resistance poses a significant threat to world health by diminishing the effectiveness of common antibiotics in treating bacterial infections. According to the WHO's Global Antimicrobial Resistance and Use Surveillance System (GLASS) report, the median prevalence of third-generation cephalosporin-resistant *Escherichia coli* (3GC-R *E. coli*) and methicillin-resistant *Staphylococcus aureus* (MRSA) across 76 countries was 42 and 35%, respectively. These high resistance rates significantly complicate the treatment of common infections [1, 2]. Furthermore, studies have revealed that antibiotic-resistant bacterial diseases are projected to directly cause over 39 million deaths globally in the next 25 years and indirectly contribute to an additional 169 million fatalities [3]. This alarming trend underscores the urgent need to explore alternatives to antibiotics for treating bacterial infections worldwide, with antimicrobial peptides (AMPs) emerging as promising options.

In this context, AMP are a class of host defense peptides characterized by their membrane-disrupting mechanisms and low potential to induce resistance. They can be widely extracted from both plants and animals [4]. Research has revealed that AMPs influence host immune responses through receptor-dependent mechanisms, such as GPR43 [5], FPR2 [6], TLR [7], and EGFR [8]. These mechanisms primarily involve the activation of signaling pathways, the release of inflammatory factors, and the modulation of immune cell functions at multiple levels. Consequently, AMPs play a crucial role

in combating infections, inhibiting tumor growth, and maintaining immune homeostasis [9]. Therefore AMPs have also become a good alternative to antibiotics.

In recent years, notable progress has been made in the clinical translation of AMPs. For instance, polymyxin B, a classical AMPs drug, has received approval for treating Gram-negative bacterial infections [10]. Additionally, daptomycin is now widely used to address complicated skin infections and bacteremia caused by drug-resistant Staphylococcus aureus [11]. In the realm of novel AMP development, NP213 (Novexatin®), a water-soluble cyclic antimicrobial peptide, has shown significant efficacy and safety against onychomycosis fungi due to its ability to effectively penetrate human nails, completing Phase II clinical trials [12]. Omiganan, a synthetic analog of bovine indolocarbocyanin, has demonstrated a superior safety and efficacy profile in patients with human tumor virus-induced genital lesions and has also been tested in phase II clinical trials [13]. These studies highlight the ongoing transition of AMPs from laboratory research to clinical application. However, several challenges remain in translating AMPs from the lab to the clinic. Natural AMPs are vulnerable to protease degradation, hemolytic toxicity, and pharmacokinetic limitations, hindering their effective delivery to target sites [14, 15]. Moreover, current delivery systems struggle to balance stability with targeted controlled release, and high production costs further limit their clinical utility.

To overcome these bottlenecks, researchers are advancing the development of AMPs by optimizing their structure and improving delivery systems [16]. For instance, the antimicrobial peptide murepavadin, which targets the outer membrane proteins of multidrug-resistant *Pseudomonas aeruginosa* and reduces host virulence through specific targeting, is currently in Phase III clinical trials [17]. In early clinical trials for the treatment of solid tumors, melittin, when combined with targeted nanoparticles, has shown advantages in controlled release and reduced hemolytic toxicity [18]. Despite challenges in clinical translation, these innovative strategies offer a significant direction for translating AMPs into clinical applications.

Current studies mostly focus on the structure and mechanism of action of AMPs, and systematic analysis of their clinical translational pathways is still insufficient. This review aims to sort out the key nodes in the clinical translation of AMPs. The biological activities of AMPs, the advantages and disadvantages existing in different delivery systems, and the problems in clinical translation are elaborated and analyzed respectively. Through the in-depth analysis of the key nodes, it provides multidimensional solutions for developing novel AMPs and promoting AMPs from laboratory to clinic.

This review begins with an overview of the mechanism of action of AMPs and highlights the advantages of their biological activities. It then compares different delivery systems for AMPs, discussing both their benefits and limitations. Finally, the review catalogs currently approved AMP-based drugs in clinical use, as well as those in various stages of clinical trials, while addressing the challenges faced in translating AMPs to clinical applications and exploring future development trends.

Mechanism of AMPs

The bioactive effects of AMPs are achieved through intricate mechanisms, and numerous studies have extensively examined and detailed their modes of action [19]. Therefore, this review briefly addresses the mechanism of action. As shown in Fig. 1, the mechanisms of action of AMPs can be primarily classified into membrane-targeting and nonmembrane-targeting mechanisms [20]. The membrane-targeting mechanism exploits the differences between bacterial microbial membranes and mammalian cell membranes, making microbial cell membranes a key target for most AMPs. Upon interacting with these membranes, AMPs accumulate on the cell surface and undergo diffusion or conformational changes. By further classifying the membrane-targeting mechanisms of AMPs based on the presence or absence of pores, they can be futher into transmembrane pore models and nonporous models [21]. The transmembrane pore models include the barrel-stave model and the toroidal pore model. The barrel-stave model is a process in which AMPs rely on an amphiphilic structure and aggregate on the bacterial cell membrane like a barrel plate around the barrel axis, forming transmembrane pores that allow the leakage of cellular contents leading to cell death, for instance, Alamethicin [22]. The toroidal pore model, on the other hand, is a process in which AMPs aggregate on the cell membrane to form a pore with themselves as the outer ring and the phospholipid head as the inner ring, which ultimately leads to the leakage of intracellular material, such as Magainin 2 [23]. And the nonporous model includes the carpet model and detergent-like model. In the carpet model, AMPs are spread on the surface of the cell membrane, and after reaching a certain concentration, they destroy the membrane structure and lead to the leakage of cell contents. In the detergent model, AMPs are inserted into the cell membrane by hydrophobicity, which ruptures the membrane and leads to cell death just like detergent dissolves grease [24]. Although each model exhibits distinct modes of action, they are interconnected in their overall effects.

Nonmembrane targeting mechanisms are primarily divided into cell wall-targeting and intracellular-targeting mechanisms. The bacterial cell wall, which is essential for survival, contains lipid II as a key component [25]. AMPs

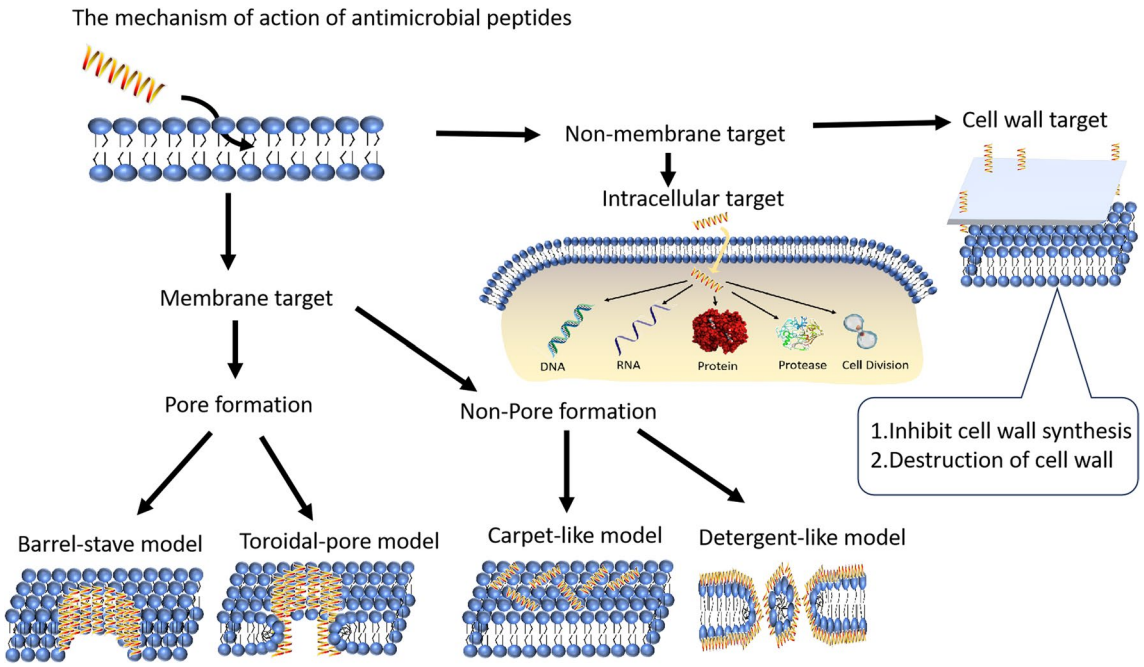


Fig. 1 Schematic diagram of the mechanistic categorization of AMPs

inhibit cell wall synthesis by binding to the pyrophosphate group or glycosyl unit of lipid II via structural domain recognition, thereby creating a spatial barrier that obstructs the synthesis process [26]. The marketed antimicrobial peptide daptomycin demonstrates bactericidal activity against Gram-positive bacteria by forming a complex with phosphatidylglycerol (PG) and lipid II in the bacterial cell membrane. This interaction leads to membrane perforation, resulting in morphological abnormalities and ultimately bacterial lysis [27]. Moreover, Nisin, as a representative antimicrobial peptide, achieves "dual-mechanism synergistic sterilization" owing to its distinctive structure. The N-terminal ring specifically binds to the pyrophosphate group of Lipid II, effectively inhibiting transglycosylase activity, while simultaneously inserting into the cell membrane via its C-terminal end to form pores [28].

Intracellular targeting mechanisms function by interfering with vital intracellular components such as nucleic acids, proteins, and proteases, disrupting their synthesis and thereby affecting fundamental cellular activities to achieve their biological effects [29]. For example, the bovine-derived AMPs indolicidin, rich in tryptophan, can embed itself within the minor groove of the DNA double helix, thereby inhibiting the topoisomerase-mediated supercoiling relaxation process [30]. arginine/proline-rich AMPs PR-39, on the other hand, degrade proteins associated with DNA replication and inhibit DNA synthesis [31].

Advantages of the biological activities of AMPs

AMPs, as antimicrobial peptides inherent in the immune response, possess a wide array of advantages in terms of their biological activities due to their complex mechanisms of action. These mechanisms include antibacterial [32], antiviral [33], antifungal [34], and antiparasitic properties [35]. Moreover, AMPs also play crucial roles in tumor inhibition [36], biofilm disruption [37], and immune regulation [38]. It lays a solid foundation for its clinical translation. Particularly in the treatment of multi-drug resistant bacteria, the antimicrobial peptide murepavadin has passed Phase III clinical trials, and its dual mechanism of biofilm disruption and rapid bactericidal activity is significantly superior to that of traditional antibiotics [17]. LL-37-Derived Peptide Induces Antitumor Effects in Melanoma Patients and Completes Phase I/II Clinical Trial in 2024 [39]. These clinical successes confirm that the multi-targeting properties of AMPs can effectively break through the limitations of existing therapies. In the following section, we will discuss in detail their core biological activities, such as anti-bacterial, anti-biofilm, and antiviral, and further understand the clinical advantages of AMPs by analyzing these molecular features.

Antimicrobial activity

Antimicrobial properties are the most remarkable properties of AMPs. The amphiphilic structure of AMPs is one of the important bases for their membrane targeting activity. Partial linear structure of AMPs exhibit an amphiphilic nature characterized by a hydrophilic N-terminus and a hydrophobic C-terminus. This structural feature arises because the C-terminus is enriched with nonpolar amino acids such as alanine, glycine, and valine, whereas the N-terminus contains a greater proportion of cationic amino acids such as arginine and lysine [40]. For example, the N-terminus of Lfcin contains multiple arginine and lysine residues, which confer hydrophilicity, while the C-terminus includes hydrophobic amino acids like valine [41]. AMPs with this type of structure bind their positively charged regions to the negatively charged components of the cell membrane surface, such as anionic lipids, lipopolysaccharides in Gram-negative bacteria, and teichoic acids in Gram-positive bacteria, through electrostatic interactions [42]. The subsequent insertion of the hydrophobic ends of AMPs into the lipid bilayer induces membrane depolarization. This then compromises the bacterial membrane integrity, leading to leakage of cellular contents and ultimately resulting in bacterial cell death [43]. This explains why such AMPs have a greater affinity for bacterial cell membranes compared to those of plants, invertebrates, and vertebrates. However, the amphiphilic conformation is not realized only in this single form, and in addition to this typical amphiphilic nature of N-terminal hydrophilic and C-terminal hydrophobic AMPs, there is also a reverse charge distribution. For example, melittin comprises 26 amino acid residues and displays an inverse topology, being hydrophilic at the C-terminus and hydrophobic at the N-terminus [44]. Some cyclic AMPs, which lack a clear distinction between their N- and C-termini, exhibit amphiphilic properties through the arrangement of amino acids within their cyclic structure. For instance, Surfactin comprises a 7-amino-acid cyclic peptide linked to a 13–15 carbon fatty acid. The fatty acid chain is highly hydrophobic, while the cyclic peptide portion displays partial hydrophilicity. This unique structure enables Surfactin to disrupt cell membranes effectively [45].

In addition to directly disrupting the bacterial cell membrane, AMPs also target the cell wall and intracellular mechanisms. Maintaining bacterial cell wall integrity is crucial for bacterial survival [46]. AMPs such as bacitracin and vancomycin can selectively bind to lipid II, thereby inhibiting cell wall synthesis [47]. Similarly human β-defensins can bind to lipid II [48]. AMPs exert their antimicrobial effects not only by interacting with cell membranes but also by targeting intracellular components. Those AMPs that engage with intracellular targets are referred to as noncleaved AMPs [49]. These membrane-nontargeted AMPs first penetrate the bacterial cells, where they accumulate and bind to biomolecules, ultimately inhibiting bacterial metabolic processes.

IARR-Anal10 synthesized by Jonggwan Park et al. [50] exhibits potent antibacterial and antibiofilm activity. Mechanistic studies have revealed that it has minimal or no effect on bacterial outer membrane permeability, membrane polarization, or membrane integrity. Instead, DNA gel-blocking analysis indicated that IARR-Anal10 binds to bacterial DNA, suggesting that its bactericidal effect is likely mediated through an intracellular mechanism. Other AMPs that kill bacteria by binding to nucleic acids include derivatives such as KW429 [51], which possesses antifungal properties, and HPA3NT330 [52], which is effective against multiple bacterial species.

The binding of AMPs to DNA significantly influences the mechanisms of bacterial resistance. Conventional antibiotics typically target bacterial cell membranes, cell walls, and specific enzymes. Bacteria can develop drug resistance through genetic mutations, alterations in the structure of target sites, and the production of inactivating enzymes [53]. In contrast, DNA-binding AMPs act on bacterial DNA, causing barriers to DNA replication and transcription and reducing the probability of bacterial resistance in several ways [54]. On one hand, DNA, serving as the core of bacterial genetic information, exhibits structural stability and resistance to mutation. DNA-binding AMPs target these conserved sites directly, making it challenging for bacteria to evade AMP action through simple genetic mutations [55]. On the other hand, DNA-binding AMPs facilitate synergistic multi-targeting, which can substantially decrease the likelihood of bacterial resistance arising from a single genetic mutation. Li et al. [56] designed a novel antimicrobial peptide using the membrane-penetrating peptide ppTG20 as a template and investigated its antimicrobial mechanism against *S. typhimurium* and *S. pyogenes*. They found that this peptide not only disrupts the cell membrane but also interacts with DNA, embedding itself between base pairs after penetrating the membrane. This dual mechanism inhibits cellular function and ultimately leads to cell death. In addition to this, DNA In addition to this, DNA-bound AMPs can circumvent membrane-associated resistance mechanisms, and because they directly target intracellular nucleic acids, they are not rendered ineffective by modifications in the bacterial membrane charge or changes in the lipopolysaccharide structure of the outer membrane [57]. It has been discovered that the antimicrobial peptide APP primarily induces cell death in *Candida albicans* by binding to DNA within the cell

membrane, halting the cell cycle at the S phase and inhibiting multiple cellular functions. Notably, this process achieves its effect with minimal disruption to the membrane integrity [58]. Although DNA-bound AMPs show promise by reducing the probability of bacterial resistance with their unique advantages, there are still many problems in practical applications, one of which cannot be ignored is that many bacteria reduce the impact of antimicrobial drugs by forming biofilms.

Anti-biofilm activity

Antimicrobial resistance (AMR) is emerging as a profound crisis that imperils the future of human health. A critical factor in this challenge is the ability of bacteria to evade antibiotic effects by forming biofilms. Biofilms are community structures encased in extracellular polymers (EPS) secreted by bacteria, composed of polysaccharides, proteins, and nucleic acids. These components hinder antibiotic penetration and modulate the expression of resistance genes via the quorum sensing (QS) system, leading to a 100- to 1000-fold decrease in antibiotic susceptibility compared to planktonic bacteria [59, 60]. The biofilm life cycle comprises four distinct stages: (1) the initial deposition and attachment of planktonic bacterial cells to a surface; (2) the strengthening of bacterial attachment along with the synthesis of the extracellular matrix; (3) the proliferation and maturation of the biofilm community; and (4) the dispersal of bacterial cells from the biofilm. Throughout these stages, bacteria undergo tightly regulated processes that involve significant shifts in metabolism, transcriptional activity, and protein expression [61]. There is a lack of clinical treatments for biofilm-associated infections, and the development of new drugs that can target biofilms is a hot research topic.

The ability of traditional antibiotics to penetrate bacterial cell membranes varies significantly and is often hindered by the bacteria's inherent defense mechanisms, encountering numerous obstacles during the penetration process [62]. For instance, β-lactam antibiotics frequently struggle to effectively cross biological membranes due to their high molecular polarity and lack of specific targeting [63]. Additionally, it has been observed that subinhibitory concentrations of aminoglycoside antibiotics can induce biofilm formation in *P. aeruginosa* and *E. coli.* This phenomenon suggests that prolonged use of traditional antibiotics may promote the development of bacterial resistance [64]. Unlike traditional antibiotics, certain AMPs have demonstrated potent and broad-spectrum activity against biofilms. One of the key advantages of AMPs is their superior ability to penetrate biofilms. For instance, the antimicrobial peptide SAAP-148 exhibits strong biofilm penetration capabilities. It effectively inhibits biofilms formed by *Staphylococcus aureus* and *Acinetobacter baumannii* at concentrations below 12.8 and 6.4 μM, respectively. Furthermore, it permeates these biofilms and kills *S. aureus* at a concentration of 51.2 μM and *A. baumannii* at 12.8 μM [64]. In addition, many conventional antibiotics are not anti-biofilm because of their poor penetration properties, whereas AMPs can inhibit one or more stages of the biofilm life cycle, effectively preventing biofilm formation through multiple mechanisms. Flagella are crucial for Vibrio cholerae biofilm formation, and polymyxin B disrupts the assembly of these flagella, leading to reduced bacterial motility and consequently impairing the bacterium's ability of biofilms [65]. Hepcidin 20 not only suppresses bacterial cell metabolism but also inhibits *Staphylococcus epidermidis* biofilm formation by reducing the production and accumulation of the biofilm's extracellular matrix [66]. LL-37 is a human cationic host defense peptide that, in addition to regulating the innate immune response and exhibiting weak antimicrobial activity, has been shown to influence biofilm formation. It reduces bacterial cell attachment, stimulates dabbling motility, and affects two major quorum sensing systems, ultimately downregulating genes essential for biofilm development [67]. Finally, Jack Wainwright et al. [68] found that traditional antibiotics are resistant to persistent cells that enter dormancy due to metabolism within the biofilm and are difficult to kill. AMPs, on the other hand, can kill persistent cells in a variety of ways, including through DNA cross-linking and inhibition of key enzymes.

With advancements in research, the advantage of utilizing the anti-biofilm activity of AMPs in combination therapy with conventional antibiotics has gained significant attention. As demonstrated by Duan et al. [69] co-administering the antimicrobial peptide Pt5-1c with traditional antibiotics such as benzathine, vancomycin, streptomycin, and azithromycin exhibited synergistic effects against three MDR bacteria growing as biofilms both in vitro and in vivo. This finding suggests the potential of using certain AMPs as adjuvants to enhance the efficacy of conventional antibiotics and to address the challenge of drug-resistant bacterial infections in clinical settings.

Antiviral activity

In addition to their antimicrobial activity, AMPs exhibit antiviral effects through several mechanisms: (1) inhibiting viral entry and cell-to-cell transmission by interacting with acetylheparin sulfate; (2) blocking viral entry via interactions with specific cellular receptors; (3) preventing viral entry through binding to viral glycoproteins; (4) interfering with membranes or viral envelopes; and (5) stimulating host cell antiviral responses or inhibiting viral gene expression [70]. Studies have shown that these

antiviral activities are frequently associated with the processes of viral adsorption and entry [71]. APB-13 inhibits the expression of the N protein gene of transmissible gastroenteritis virus (TGEV) at both the transcriptional and translational levels, thereby suppressing TGEV proliferation [72]. Similarly, epinecidin-1, an antimicrobial peptide, inhibits viral replication by reducing mRNA and protein expression, demonstrating in vitro activity against foot-and-mouth disease virus [73]. LL-37 inhibits inhibitory effects on a wide range of viruses, including human immunodeficiency virus (HIV) [74], influenza A virus (IAV) [75], vaccinia virus (VV) [76], herpes simplex virus (HSV) [77], dengue virus (DENV) [78], and Zika virus (ZIKV) [79]. This is achieved this by disrupting viral membranes, inhibiting DNA replication, and stimulating the antiviral mechanisms of host cells.

Anti-inflammatory activity

Inflammation is a defensive response initiated by harmful stimuli and inflammatory factors. This process, which encompasses various physiological and pathological mechanisms, aims to maintain homeostasis, albeit at the cost of a temporary reduction in tissue function [80]. Inducers are signals that initiate inflammatory responses by activating specialized sensors. These sensors then trigger the production of specific mediators that alter the functional state of tissues and organs. Inducers are generally classified into exogenous and endogenous types. Exogenous inducers can be further divided into microbial and non-microbial categories, such as pathogen-associated molecular patterns (PAMPs) and virulence factors. Endogenous inducers, on the other hand, are signals generated by stressed, damaged, or dysfunctional tissues [81]. The role of AMPs in the immune process, as part of the innate immune response in all organisms, is highly complex. The anti-inflammatory activities of AMPs reported to date involve preventing the binding of inflammation inducers to their respective sensors and inhibiting the regulation of inflammation-related signaling pathways and transcription factors through two distinct mechanisms [82].

The most potent virulence factors of gram-negative and gram-positive bacteria are lipopolysaccharides (LPSs, also known as endotoxins) and lipoproteins (LPs), respectively. These factors are recognized by distinct pattern recognition receptors (PRRs): LPS signals through Toll-like receptor 4 (TLR4), whereas LP signals through Toll-like receptor 2 (TLR2) [83]. In vivo, LPS binds to LBP and is then transferred from CD14, an anchoring protein, to TLR4. This interaction activates the TLR4 pathway, leading to the expression of inflammatory factors [84]. AMP exerts its anti-inflammatory effects through three mechanisms: (1) neutralizing LPS, (2) inhibiting the binding of LPS to LBP, and (3) competitively binding to LPS to block its transport [82]. In vitro, AMPs with strong positive cationic properties can modulate the immune response by binding to LPS [85]. Schromm et al. [9] used techniques such as SAXS and AFM to determine the three-dimensional structure, surface charge, and transport of LPS when modulated by LL-32 and PMB. There findings demonstrated that both LL-32 and PMB can neutralize LPS in vitro. Gutsmann et al. [86] used biophysical techniques to demonstrate that a novel synthetic antilipopolysaccharide peptide (SARP) neutralizes LPS by transforming the lipid A portion from its "endotoxic conformation" (cubic aggregate structure) into an inactive multilayered structure. Theese authors further showed that SARP has a greater binding affinity for LPS than does LPS-binding protein (LBP). Preclinical studies by Lena Heinbockel et al. [87] demonstrated that Pep19-2.5 exhibits high endotoxin neutralization efficiency both in vitro and in a mouse model of bacteremia. Moreover, the AMPs CAP18 and CAP11 from the cathelicidin family not only possess LPS-binding activity but also inhibit inflammation by binding to the cell surface of CD14 and preventing LPS-cell interactions, an effect confirmed in a mouse model of endotoxic shock [88].

In addition to inhibiting the activation of inflammatory signaling pathways upstream, AMPs can also act on inflammatory signaling pathways to exhibit inhibitory activity. The TLR signaling pathway, the NF-κB pathway, and the MAPK pathway are a few of the critical signaling pathways associated with the regulation of inflammatory signal transduction [89, 90]. Inflammatory responses induced by exogenous factors typically initiate with the activation of the TLR signaling pathway. Once PAMPs are recognized and bound by TLRs, this interaction triggers the activation of downstream pathways, including the NF-κB and MAPK pathways [91]. The well-known NF-κB pathway plays a crucial role in regulating the expression of various inflammatory responses. It primarily governs the expression of key proinflammatory mediators such as TNF-α, IL-6, IL-1β, and inflammatory chemokines [92]. The MAPK pathway encompasses C-Jun amino-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38MAPK), and extracellular signal-regulated kinase (ERK). Activation of this pathway leads to the phosphorylation of JNK, p38, and ERK, thereby promoting inflammation [93]. Interestingly, AMPs has either proinflammatory or anti-inflammatory effects depending on the expression levels of inflammatory factors and cytokines at the site of inflammation [94].

SET-M33D is a synthetic peptide that not only neutralizes LPS and LTA but also downregulates the expression of various proinflammatory factors, including cytokines (TNF-α and IL-6), enzymes (cyclooxygenase-2 (COX-2),

and nitric oxide synthase (iNOS)), chemokines (macrophage inflammatory protein-1 (MIP-1), IP10), and transcription factors (NF-κB). This multifaceted action results in a potent anti-inflammatory effect.

WALK11.3, designed by Shim et al. [95] functions as an anti-inflammatory agent by specifically inhibiting TLR4 endocytosis. This peptide concurrently suppresses the expression of inflammatory mediators such as NO, COX-2, IL-1β, IL-6, IFN-β, and TNF-α. Mt6, developed by Kong et al. [96] along with its D-enantiomer D-Mt6, inhibits LPS-induced activation of mitogen-activated protein kinase in inflammatory macrophages, thereby exerting an anti-inflammatory effect.

AMPs drug delivery system study

The diverse biological activities of AMPs make them suitable for a wide range of disease applications. However, challenges such as short residence times at the lesion site and susceptibility to degradation by the environment and proteases pose significant obstacles to the clinical translation of AMPs. These limitations can be effectively addressed through the rational design of AMP delivery systems. For example, successful products such as polymyxin B liposomes have already combined drug delivery systems with AMPs. Suitable delivery systems for AMPs include hydrogels, self-assembled AMPs, nanoparticles, and others, which should be chosen basis of the characteristics of each system and the specific application context.

Based on antimicrobial peptide modifications

Natural AMPs exhibit instability, toxicity, and limited bioavailability, which restrict their clinical application. For instance, LL-37 plays an important role in the innate immune defense against bacterial infections, however, the cytotoxicity of LL-37 to eukaryotic cells limits its clinical application [97]. In recent years, significant advancements have been made in enhancing the stability, reducing the toxicity, and increasing the bioavailability of natural AMPs. PEGylation, a widely used technique, effectively improves stability by prolonging the half-life of AMPs in vivo through conjugation with PEG. Additionally, the hydrophilic nature of PEG increases the solubility and stability of AMPs [98, 99]. Cyclization is another promising approach, which modifies the spatial conformation of AMPs to form ring-like structures. This not only enhances resistance to proteases but also reduces toxicity in some instances [100]. These stability-enhancing methods are opening up new possibilities for the clinical application of AMPs.

AMPs feature a structure rich in consecutive cleavage elements and arginines, endowing them with several distinctive properties such as a positive net charge, hydrophobicity, and amphiphilicity [101]. Leveraging these characteristics, researchers have modified and optimized AMPs to increase their therapeutic potential. These modifications aim to increase antimicrobial activity, improve serum stability, and reduce toxicity. The most common modification method for AMPs involves altering their sequences. By adding, deleting, or substituting amino acid residues within AMP sequences, this approach enhances their biological activity [102]. However, in the pursuit of stability enhancement, the trade-off between stability and activity must be emphasized. When the AMP sequence is modified to improve stability, while there may be benefits such as increased serum stability and improved protease resistance, there is also a potential risk of loss of activity. Denise Meinberger et al. [103] modified the sequence of the antimicrobial peptide CLEC3A, resulting in WRK-30. This altered peptide demonstrated enhanced antimicrobial activity against both *Staphylococcus aureus* and MRSA, but also exhibited reduced cytotoxicity toward eukaryotic cells. By incorporating a sulfonyl-γ-AA building block into Feleucin-K3, Guo et al. [104] discovered that the serum stability of CF3-K11 was enhanced by 8–9 times. Additionally, compared with conventional antibiotics, CF3-K11 exhibited a faster bactericidal effect and a lower propensity for resistance development. Furthermore, substituting L-amino acids with D-amino acids in AMPs can significantly enhance protease stability and improve pharmacokinetic properties. Chen et al. [105] employed a D-arginine substitution strategy to replace L-amino acids in the antimicrobial peptide HBcARD, resulting in a D-enantiomer that retained equivalent antimicrobial activity while exhibiting reduced hemolytic toxicity. In a mouse model of *S. aureus* infection, the D-antimicrobial peptide demonstrated a markedly higher survival rate compared to the L-antimicrobial peptide (40 vs. 100%). A study by Jlenia Brunetti et al. [106] also found that D-amino acid substituted AMPs possess both bactericidal and anti-inflammatory activities, and exhibit greater resistance to bacterial proteases.

Although modifications to the sequence not only enhanced activity but also reduced toxicity in the above studies, this is not universally the case. Excessive modifications and substitutions of amino acids or irrational modifications can make AMPs cytotoxic and immunogenicity increase. Lu et al. [107] synthesized a derivative of the cationic AMP Pep05 (KRLFKKLLKYLRKF), named DP06, by replacing L-amino acid residues with D-amino acids. They found that in vivo, DP06 showed markedly reduced activity and increased toxicity. This study fully illustrates the negative impact of excessive modification on activity while enhancing stability. Therefore, when modifying the sequence, the degree of

modification should be precisely controlled, and the stability of the antimicrobial peptide should be improved through reasonable modification of amino acids, so that the activity of the antimicrobial peptide can be guaranteed and its stability can be improved at the same time.

Peptide cyclization is a common modification technique that enhances the protease stability and selectivity of AMPs by forming cyclic structures through head-to-tail, side-to-side, or head-to-side linkages [100, 108]. This process is typically achieved using disulfide bonds, lactones, or other methods. Neda Riahifard et al. [109] designed both linear and cyclic AMPs and discovered that the cyclic peptides exhibited significantly higher antimicrobial activity, with a minimum inhibitory concentration (MIC) of 4 μg/ml. Moreover, certain cyclic peptides exhibit the capability to penetrate cells and increase cell membrane permeability [110]. Fang et al. [111] developed an aromatic cross-linked cyclic peptide that can traverse biological barriers while enhancing protein hydrolysis stability. Deendayal Mandal et al. [112] designed an amphiphilic homochiral L-cyclic peptide, which subsequent studies revealed to be non-cytotoxic and to possess robust cell-penetrating properties. Additionally, this amphiphilic L-cyclic peptide proved to be highly efficient with exceptional cell penetration ability. In conclusion, the cyclization of AMPs is an effective modification strategy. Many clinically used AMPs drugs, such as bacitracin, polymyxins, tyrothricin, and daptomycin, are cyclic peptides. Although significant progress has been made in developing cyclic peptides, the design and synthesis of cyclic AMPs remain challenging areas that require further research [113].

The glycation of AMPs primarily involves the covalent bonding of polysaccharides to these peptides, which subsequently facilitates peptide folding and enhances their diversity. Furthermore, the glycation of AMPs improves their amphiphilicity and increases their stability against proteases [114, 115]. Glycosylation can be categorized into O-glycosylation, S-glycosylation, C-glycosylation, and N-glycosylation. O-glycosylated peptides are generated by attaching a glycosidic moiety to threonine or serine residues. Drosocin is a 19-amino acid glycosylated AMPs and belongs to the proline-rich group. Lele et al. [116] demonstrated that O-monoglycosylated Drosocin completely eradicated bacteria within 40 min, whereas non-glycosylated Drosocin failed to fully eliminate *E. coli* even after 360 min. This indicates that O-glycosylated Drosocin exhibits a lower minimum inhibitory concentration (MIC) and faster bactericidal activity. N-glycosylation primarily involves the linkage of glycan chains to asparagine residues. In AMPs, N-glycosylation influences properties such as rigidity and solubility. Attila Totorella et al. [117] investigated the effects of N-glycosylation on the antimicrobial peptide LL-III and discovered that N-glycosylation of LL-III reduced the hydrolysis rate of the AMPs, enhanced their protease resistance, while preserving the original antimicrobial activity and mechanism of action.S and O belong to the same family, which means their chemical bonding properties are somewhat similar. As a result, many researchers have begun to use S-glycosylation as an alternative to O-glycosylation. S-glycosylation primarily involves cysteine residues. Sublancin, an S-glycosylated peptide, has demonstrated relaxed substrate specificity and remarkable stability [118]. However, Chen et al. [119] found that S-glycosylation and O-glycosylation exhibit significant kinetic differences, resulting in distinct glycosylation effects. Comparisons revealed that O-glycosylation markedly enhances protein hydrolytic stability, thermal stability, and cellulose affinity, whereas S-glycosylation only improves thermal stability. This may explain why S-glycosylation is less common. C-glycosylation enhances the interaction between AMPs and reduces their cytotoxicity. Eduardo et al. [120] C-glycosylated HSP1-NH to generate chylaseptin-P1 and found that this glycosylated antimicrobial peptide exhibited greater bilayer-disrupting activity. Its antifungal efficacy was significantly improved, showing eight times higher activity in inhibiting ergosterol biosynthesis compared to the unglycosylated peptide.

In addition to sequence alteration, cyclization, and glycosylation, other modifications such as PEGylation and lipidation also play significant roles. Each of these modifications exerts distinct effects on the therapeutic potential of AMPs (as detailed in Table 1). Although modifying AMPs can significantly enhance their antimicrobial activity and reduce hemolysis, such modifications should not be carried out blindly. For instance, the cytotoxicity of AMPs is closely related to their hydrophobicity and amphiphilicity, which can be effectively mitigated through PEGylation. AMPs composed of shorter linear sequences and L-amino acids have lower production costs but are more susceptible to protease-mediated hydrolysis. In contrast, cyclic structures and D-amino acids improve peptide stability, at a higher production cost [110]. Wang et al. [121] discovered that modifying the antimicrobial peptide $F_{2,5,12}W$ through mPEG coupling, amino acid sequence alteration, and disulfide bond cyclization significantly enhanced its plasma stability and decreased its hemolytic activity.

However, these modifications also led to a reduction in the peptide's antimicrobial efficacy, thereby limiting its potential for clinical application. For the chemical modification of AMPs, finding a balance between stability, antimicrobial activity, hemolysis, production cost, and other factors presents a significant challenge. Dongin Kim et al. [122] devised the design of a monomeric

Table 1 Advantages and disadvantages of surface modification methods for AMPs and options to overcome them

Methods of chemical modification	Classifications	Advantages	Disadvantages	Ways to overcome	Example	Refs.
Changes in amino acid sequence	Additions (N-/C-terminal acetylation, etc.) Substitution (replacement of D-amino acids, uncommon amino acids, etc.) Removing	Improved stability and pharmacokinetic properties of AMPs against proteases Improve peptide biocompatibility and bioavailability Improve target selectivity Cell membrane penetration (transportation of cargo) Stabilization of a-helix weakness Improvement of antimicrobial activity High-cost synthesis	High-cost synthesis Increased epitope rigidity of AMPs Net charge neutralization of AMPs epitopes Hydrophobic enhancement, toxic to mammalian cells May disrupt antimicrobial activity of AMPs Some alterations limited by their short length	Incorporation of a single Aib residue at the N-terminus provided significantly enhanced plasma stability and higher in vivo activity Incorporation of unnatural amino acids and other chemical modifications to increase cost-effectiveness and ensure structure–function relationships required for antimicrobial therapy Effective antimicrobial drugs can be developed by leveraging database resources. Since the optimized sequences may have a high degree of sequence homology with host defense peptides	SAAP 148 6K-F17	[124, 125]
Cyclize	/	Enhances peptide hydrolytic stability Improve target selectivity	Time-consuming and costly synthesis Peptide cyclization results are not easily predictable	Incorporation of unnatural residues in cyclic peptides. And the success rate can be improved by AI technology for assisted calculation of sequence and structure	Polymyxin Daptomycin	[100, 108]
Glycosylation	O-Glycosylation N-Glycosylation S-Glycosylation C-glycosylation	High targeting Improved AMP stability Altered half-life Increases the diversity of proteins and/or peptides and extends their functional range	May reduce original antimicrobial activity Hydrophilic glycosylation reduces hydrophobicity of AMPs	Two different chemical modifications are combined in a proposed peptide The peptide can be expressed by recombinant bacteria and then glycosylated by plasmid-encoded enzymes High-throughput screening analysis in combination with gAMP libraries	Bactenecin Datucin Drosocin	[114]
Lipidation	N-Acylation S-Acylation	Improves protease enzyme stability Regulates hydrophobicity and self-assembly propensity of AMPs	Reduced therapeutic index due to toxicity to healthy mammalian cells	Appropriate modulation of fatty acids incorporated into AMP sequences resulting in cost-effective therapeutic agents Low stability in physiological environments	Caspofungin Anidulafungin	[126]

Table 1 (continued)

Methods of chemical modification	Classifications	Advantages	Disadvantages	Ways to overcome	Example	Refs.
PEGylation	/	Improved half-life Minimal toxicity and immunogenicity Improved stability and biocompatibility	Damaged peptide structure May reduce original antimicrobial activity Reduced binding affinity of AMP due to spatial site resistance of PEG	Development of ultrashort sequences to avoid increase in molecular weight Synergize with other antimicrobial agents to improve the effectiveness of these molecules in animal infection models	AMP OM19r-8 AMP Ac-CGGP9-PEG	[98, 99]

pseudo-isolated α-helix (mPIH) system that does not require any covalent chemical modification of proteins, and the optimal mPIH showed more than 100-fold increase in target selectivity, and the study demonstrated that mPIH can become a promising protein-based platform for developing stabilized α-helix pharmaceuticals and based on the inspiration of its study, whether it can be applied to the design of AMPs. With the emergence of AI technology, it is now possible to analyze vast amounts of antimicrobial peptide data and predict the relationship between their structure and function. This approach can optimize the structure of AMPs, enabling a more effective balance between antimicrobial activity, side effects, and stability [123].

Hydrogel-based drug delivery systems

With the advancement of drug delivery strategies, these approaches have demonstrated superior characteristics compared to the structural modifications of AMPs. Structural modifications are not only unpredictable in their outcomes but also entail high production costs [127]. In recent years, hydrogels have garnered significant attention in wound healing. This is primarily due to their ability to fill irregular wounds, minimize invasiveness, and maintain high water retention, which enhances wound moisture and reduces surrounding temperature, thereby improving patient compliance. Furthermore, composite hydrogels created from multiple polymers can introduce novel properties to the gels [128].

There are numerous hydrogel materials available for the delivery of AMPs. Günnur Pulat et al. [129] developed a gelatin methacrylate (GelMA) hydrogel and immobilized the antimicrobial peptide P9-4 through photo-induced coupling and EDC/NHS chemistry. They found that the AMP-loaded hydrogel exhibited greater antimicrobial activity against *P. aeruginosa* and *MRSA* compared to administration by immersion or simple mixing. However, covalent immobilization of AMPs on polymer hydrogels often results in reduced antimicrobial efficacy. Additionally, the synthesis process is complex, time-consuming, labor-intensive, and may involve cytotoxic cross-linking agents. Therefore, many researchers have utilized polysaccharide and protein polymers to develop hydrogels. Guo et al. [130] created a dual-network hydrogel composed of collagen-peptide-functionalized carboxymethyl chitosan, sodium methacrylate, and alginate, which was further combined with the antimicrobial peptide SALSP. This hydrogel demonstrated excellent antimicrobial properties, effectively controlling wound bacterial infections by the third day. Additionally, the hydrogel served as a delivery system for the antibacterial peptide, enhancing its efficacy in managing wound infections.

However, for these types of hydrogels, the manufacturing process often becomes more complex due to the addition of a second polymer, salt ions, or extra functionalization steps. In contrast, DNA-fabricated hydrogels present several more straightforward advantages. Sybil Obuobi et al. [131] leveraged the high affinity between anionic DNA nanostructures and cationic AMPs to develop DNA hydrogels, as illustrated in Fig. 2. The resulting hydrogels demonstrated nuclease-sensitive degradation, controlled drug release, and significant antimicrobial activity against *E. coli*, susceptible *S. aureus*, and *MRSA* infections. Antimicrobial assays revealed a 1-log reduction in *E. coli* colonies at 32×MIC. For both susceptible and resistant *S. aureus*, no colonies were observed at 16×MIC and 32×MIC. In vivo studies further showed that the DNA hydrogels loaded with AMPs exhibited rapid anti-inflammatory effects and promoted wound healing.

AMPs can also self-assemble to form gels without the need for cross-linking or drug loading. PAF26, a cationic antimicrobial peptide with amphiphilic properties, can be induced to self-assemble into molecular hydrogels by adjusting the pH. As illustrated in Fig. 3, Cao et al. [132] discovered that PAF26 hydrogels exhibit shear-thinning behavior and are injectable. Antimicrobial studies confirmed that these self-assembled hydrogels demonstrate excellent antimicrobial activity against *C. albicans*, *S. aureus*, and *E. coli*, achieving nearly 100% killing efficiency.

Hydrogel-based drug delivery systems offer numerous advantages, leading to their widespread use in treating various lesions. However, when applied clinically, AMPs hydrogels still face several limitations, such as tissue toxicity, hemolysis, and the rapid release of the hydrogel itself. Additionally, their inability to support systemic drug delivery restricts clinical applications primarily to local treatments [133]. However, from a long-term clinical translation perspective, investigating its biocompatibility and potential for systemic drug delivery is especially crucial to overcome existing limitations and expand its application scope.

In terms of biocompatibility, hydrogels are generally recognized for their superior biocompatibility due to their ability to mimic the extracellular matrix, thereby providing an optimal microenvironment that supports cell adhesion, proliferation, and differentiation. However, when hydrogels are loaded with AMPs, the biocompatibility can be influenced by the characteristics of the matrix materials. These materials, which can affect overall biocompatibility through factors such as purity, degradation products, and inherent properties [134]. Victoria O. Fasiku et al. [135] found that the CS-HP-P gel exhibited cytotoxic effects and induced cellular damage

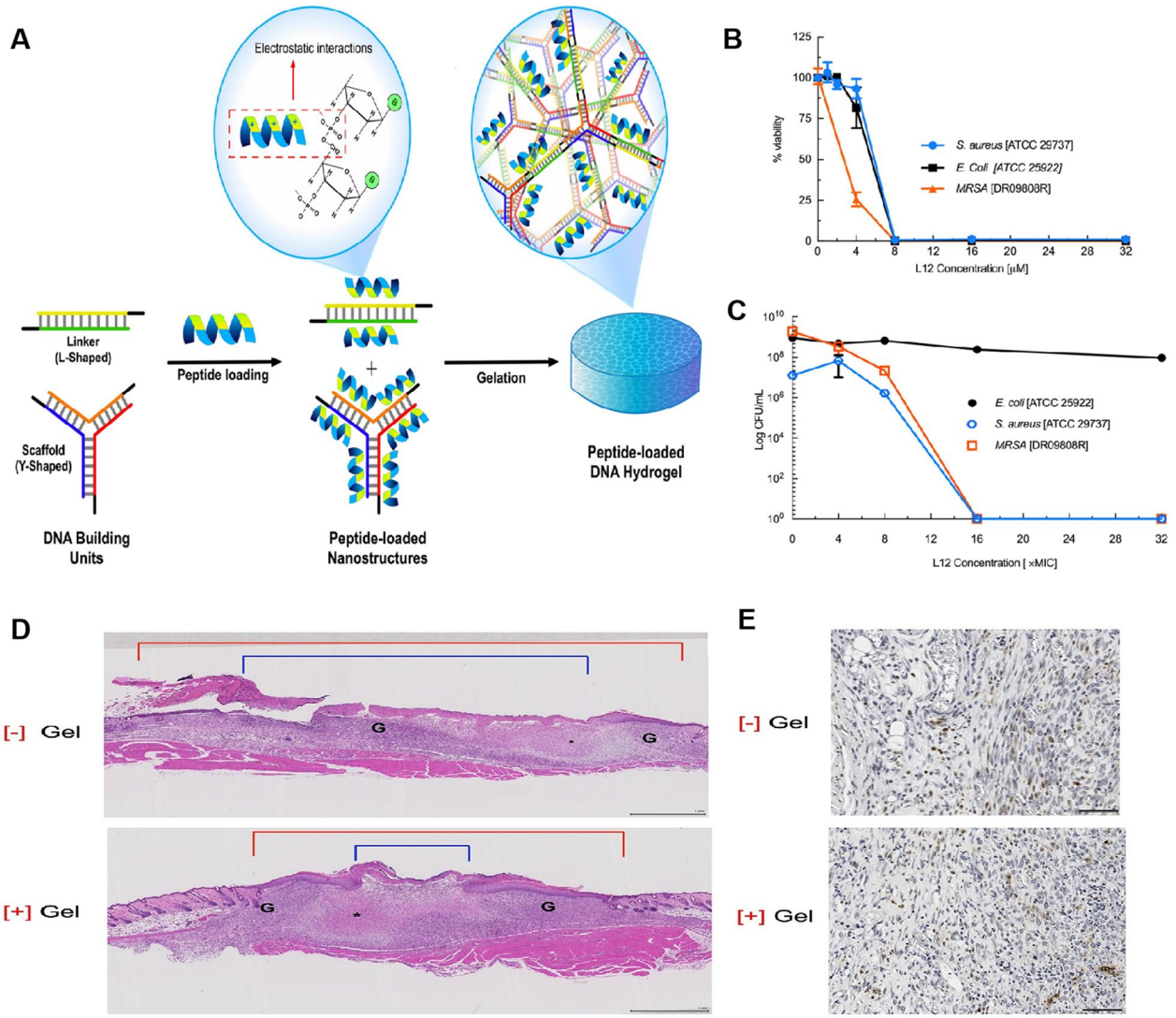


Fig. 2 **A** Schematic representation of the preparation process of L12-loaded DNA hydrogel. **B** The MIC method was used to evaluate the viability percentages of *S. aureus*, *E. coli* and MRSA after treatment with antimicrobial peptide L12 solution. **C** In vitro killing efficiency evaluation of L12 loaded DNA hydrogels against S. *aureus*, *E. coli* and *MRSA* over 24 h (n = 3). **D** Representative H&E staining of wound excision tissues. **E** TUNEL stain section of skin tissues treated in the control group (–Gel) and the DNA hydrogel treatment (+ Gel) group after 10 days. Scale bar: 100 μm (from Sybil Obuobi et al. [131]). The image has been reproduced with permission from publisher

at concentrations exceeding 100 μg/mL. Lei et al. [136] developed Gel-Col@AMP2-Ce6, which demonstrated minimal toxicity to L929 cells in vitro at a concentration of 16 μM. However, in vivo studies revealed that the Ce6 component of the gel material also caused some tissue toxicity in animals. On the other hand, unstable binding between AMPs and hydrogels can result in premature release or aggregation of AMPs in vivo, potentially compromising biocompatibility [137]. Therefore, it is crucial to design hydrogels that can precisely control the release of AMPs. Xiong et al. [138] utilized electrostatic interactions to develop an antimicrobial hydrogel by combining a negatively charged antimicrobial peptide, Fmoc-FFWDD-OH, with a positively charged poly(hex amethylenebis(methylenebis(guanidinium))hydrochloride) (PHMB). This hydrogel releases PHMB efficiently under acidic conditions but significantly slows the release rate in weakly alkaline environments, thereby achieving controlled and environment-specific delivery of AMPs.

In addition to the biocompatibility of the gel, its potential for systemic drug delivery has been a focus of research on this material. Jiang and others then developed hydrogel patches that can be implanted in the body to treat heart-related injuries and diseases [139].

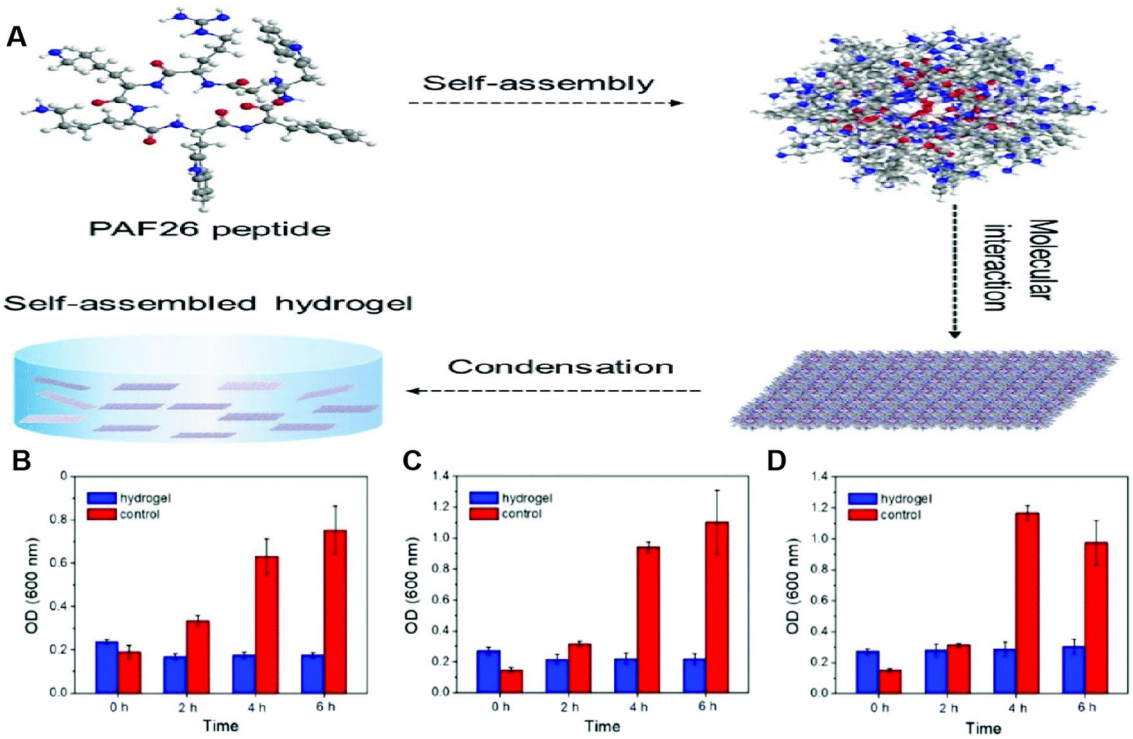


Fig. 3 **A** Mechanism of self-assembly of the antimicrobial peptide PAF26 into hydrogels. **B**, **C** PAF26 hydrogels were cultured in agar medium containing *C. albicans* (**B**), *S. aureus* (**C**), and *E. coli* (**D**) for 6 h with OD600 nm values. (From Cao et al. [132]). The image has been reproduced with permission from publisher

However, current hydrogel-based delivery systems for AMPs encounter numerous challenges in systemic drug delivery. One key issue is that the shape of the hydrogel significantly influences its distribution and circulation time within the body. Hydrogels with larger particle sizes tend to be rapidly cleared by the reticuloendothelial system, hindering their ability to reach the target site effectively [140, 141]. Secondly, the stability of hydrogels in blood circulation and their degradability are critical concerns. Hydrogels must maintain structural integrity during application while preventing adverse reactions such as blood clot formation. Finally, achieving precise targeting of hydrogels after systemic administration remains a significant challenge [142]. Numerous researchers have been developing targeted and responsive hydrogels to enhance their affinity for specific tissues or cells. For instance, Wang et al. [143] designed a ROS-responsive TSPBA-PVA hydrogel that enables targeted drug release at sites of myocardial injury, thereby improving treatment for myocardial infarction. In conclusion, hydrogels as drug delivery systems for AMPs offer unique advantages. While more studies focus on systemic drug delivery using hydrogels, true clinical realization remains challenging. Nonetheless, this highlights key areas for future research in developing hydrogel-based AMPs drugs.

Lipid-based drug delivery systems

The lipid-based material delivery system represents the first drug delivery platform approved by the FDA for clinical applications. The materials utilized in this system exhibit superior safety and biocompatibility compared to alternative delivery methods, along with key advantages such as modifiability and the capacity to transport both hydrophobic and hydrophilic molecules. These attributes make lipid-based drug delivery systems an excellent choice for administering AMPs [20]. Lopez-Berestein et al. [144] found that liposomal amphotericin B at similar concentrations was equally effective as the free drug. Animals treated with a higher concentration of liposomal amphotericin B (4 mg/kg) exhibited prolonged survival. Thus, the study suggests that encapsulating amphotericin B in liposomes enhances the therapeutic index. Moreover, polymyxins have limited applications due to their nephrotoxicity. Until polymyxin B was encapsulated in liposomes, it was found that it did not affect the antimicrobial activity of AMPs and reduced its nephrotoxicity [145]. Subsequent investigations demonstrated that liposomal formulations of polymyxin B exhibited superior permeability against *P. aeruginosa* aggregates, leading to a lower MIC value compared to unencapsulated AMPs [146]. And Li et al. [147] found that all of

the mucin-treated mice died within 24 h of infection, whereas 50% of the mice in the mucin liposome-treated group survived for up to 96 h. All of these examples above demonstrate that liposomes can be effective in successfully reducing toxicity while maintaining efficacy. Moreover, liposomes can be modified for targeted drug delivery. For instance, Cui et al. [148] successfully achieved targeted delivery of polymyxin B by modifying liposomes. While oral administration remains the preferred route for drug delivery, oral drugs must traverse the gastrointestinal tract and other organs upon entering the body. This is particularly challenging for AMPs, which exhibit very low oral bioavailability, thereby limiting their therapeutic potential. However, oral delivery of AMPs is anticipated to be achieved through the surface modification of liposomes. Werner et al. [149] utilized a cyclic cell-penetrating peptide to modify liposomes, which were then used to encapsulate a vancomycin derivative, FU002. They found that this liposomal formulation significantly enhanced the oral bioavailability of FU002 while preserving its high antimicrobial activity. In vivo studies demonstrated that the reduction in bacterial load in the liver was comparable between orally administered FU002 liposomes and intravenously administered free FU002 (as shown in Fig. 4). This finding provides valuable insights for the development of other related peptide-based formulations.

Although it is commonly thought that bacteria do not develop resistance to AMPs, evidence has emerged showing that insensitivity to these peptides can occur, as with vancomycin-resistant Enterococcus faecalis (VRE) during infections. Research indicates that bacteria may indeed develop resistance to AMPs in a manner similar to traditional antibiotics [150]. Consequently, utilizing a drug delivery system that combines multiple agents could be an effective strategy to reduce the prevalence of drug-resistant bacteria. Li et al. [151] designed a new liposomal formulation encapsulating daptomycin and clarithromycin in an optimal ratio, and found that the combined liposomal formulation showed higher antimicrobial activity

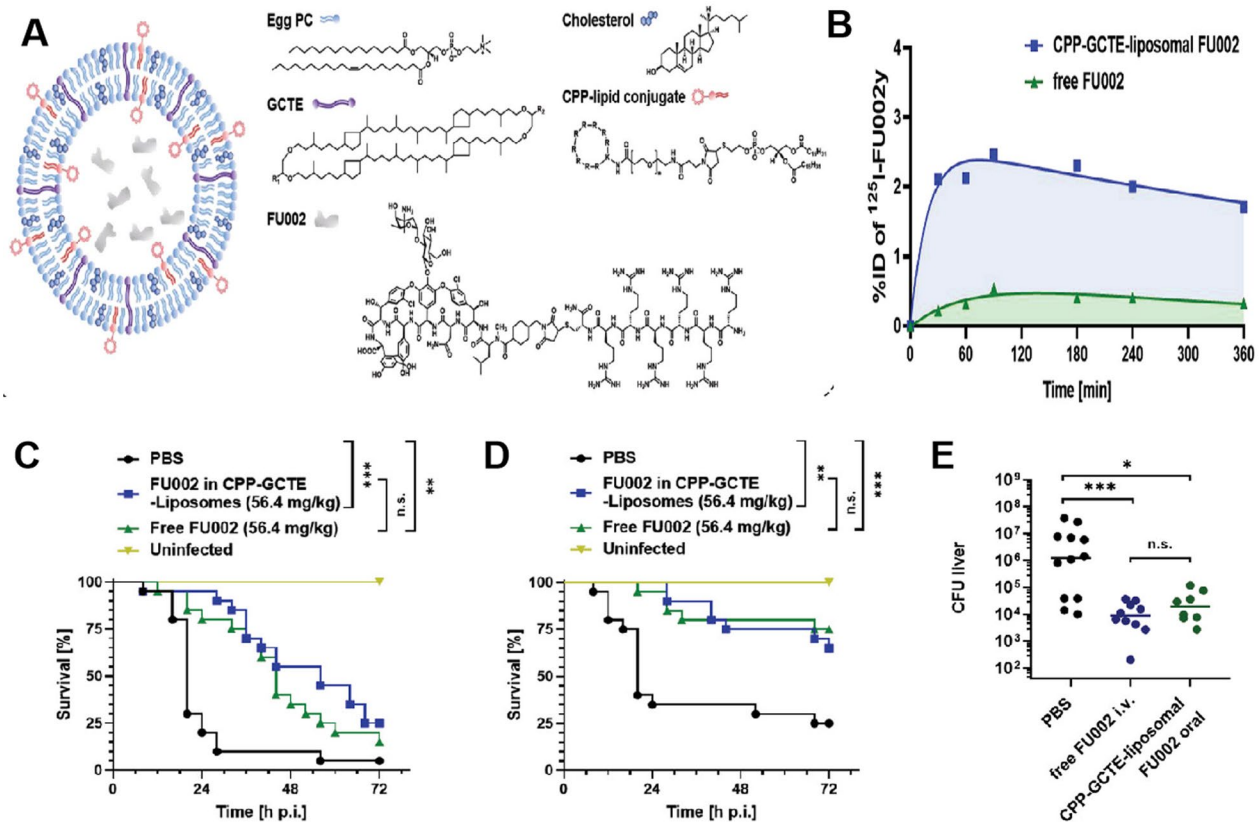


Fig. 4 **A** Schematic diagram of the structure and composition of oral FU002 liposomes. **B** After oral administration of CPP-GCTE-iposomes FU002 (AUC0-360 = 732.2) and free form (AUC0-360 = 136.1), the levels of FU002 in the blood were measured, values presented as mean; n = 3. **C** The survival rate of larvae infected with MRSA after treatment with liposomes and free FU002. **D** The survival rate of larvae infected with Enterococcus faecalis after treatment with liposomes and free FU002. **E** After mice were infected with *MRSA*, PBS, free FU002, and liposomes were administered to evaluate the antibacterial treatment effect through liver CFU (from Werner et al. [149]). The image has been reproduced with permission from publisher

and prolonged the survival of infected mice in vitro and in vivo than the individual drug liposomes, and also reduced the risk of bacterial resistance. In addition, the modification of liposomes not only enhances the antibacterial activity of the antimicrobial peptide, but also improves its effect on drug-resistant bacteria. Moreover, lipophilic adjuvants can enhance the efficacy of colistin. Carla Faivre et al. [152] designed nanoliposomes loaded with (E, E)-farnesol and myristic acid, encapsulating colistin (CST). They discovered that these nanoliposomes significantly reduced the MIC of CST needed to inhibit bacterial growth, increasing in vitro efficacy by at least 16-fold. Additionally, this formulation effectively overcame mucin resistance in *A. baumannii*.

Drug resistance poses a significant challenge not only in antimicrobial therapy but also in cancer treatment. While some AMPs exhibit anticancer properties, their potential toxicity when used alone limits their effectiveness. To address multidrug resistance (MDR) and minimize cytotoxicity, developing carrier systems with unique mechanisms of action and selective targeting of cancer cells has become a top priority. Lu et al. [153] co-encapsulated AMPs Chrysophsin with doxorubicin in polyethylene glycol-modified liposomes and discovered that these drug-loaded liposomes exhibited significantly enhanced cytotoxicity against cancer cells compared to the free drugs ($P<0.05$). This researches find advantages such as selective tumor-killing activity, bypassing traditional multidrug resistance mechanisms, and demonstrating a cumulative effect in combination therapy. Zhu et al. [154] encapsulated the antimicrobial peptide ferredoxin 2–3 along with doxorubicin in polyethylene glycol-modified liposomes. They found that these drug-loaded liposomes demonstrated significantly greater inhibition of cancer cells compared to doxorubicin alone ($56.16 \pm 4.61\%$, $P<0.05$). Further studies revealed that this enhanced efficacy was achieved through multiple mechanisms, including exocytosis, autophagy, and/or necroptosis pathways, mediated by efflux pump proteins and complex regulatory networks. This approach not only improved the effectiveness of chemotherapy but also effectively inhibited the growth of HeLa cancer cells, overcoming multidrug resistance in cervical cancer cells. In addition, Ron-Doitch et al. encapsulated the antimicrobial peptide LL-37 into liposomes and observed that while 20 μM of free LL-37 led to only 20% cell survival after 24 h, liposomal LL-37 at a concentration of 310 μM maintained over 60% cell survival during the same period. Further studies on antiviral activity revealed that the free LL-37-treated group exhibited a narrower antiviral profile (EC50 = 18.7 μM; CC50 = 37.3 μM). In contrast, liposomal LL-37 demonstrated a broader, bell-shaped antiviral profile with notable cytotoxicity at concentrations exceeding 25 μM (EC50 = 4.2 μM; CC50 = 43.8 μM). These findings suggest that liposomal formulations can preserve efficacy while effectively reducing toxicity. Collectively, these cases highlight the promising potential of liposomal formulations in developing safe and effective AMP-based therapies.

In conclusion, these studies and the currently marketed liposomal drugs have demonstrated the excellent properties and promising clinical applications of liposomes, establishing them as a reliable drug delivery system. However, when applied clinically, liposomes must address certain limitations, such as low in vivo stability, the lack of effective sterilization methods, and challenges in scaling up the production process. These issues have impeded the industrialization of liposomal drug delivery systems. Therefore, overcoming these challenges is crucial for the successful clinical translation of liposomal formulations [155].

Nanomaterial-based drug delivery systems

Nanomaterials have emerged as a significant research focus in recent years, attributed to their diminutive size, substantial specific surface area, targeting capabilities, and functionalization potential. The encapsulation of AMPs within nanomaterials holds immense promise. This approach not only enhances the stability and efficacy of AMPs while reducing toxicity to host cells but also combats infections caused by drug-resistant bacteria [156]. Nanomaterials engineered for AMP encapsulation primarily consist of inorganic, polymeric, and lipid-based materials.

Inorganic nanomaterials are novel materials characterized by their stable structures and advantageous physical properties. These materials can primarily be divided into two categories: non-metallic (such as nano-silica and nano-titanium dioxide) and metallic (like gold and copper) [157]. Notably, non-metallic nanomaterials often consist of silica nanoparticles. For instance, Guo et al. [158] encapsulated the antimicrobial peptide polymyxin B within hyaluronic acid-modified silica nanoparticles, successfully developing antimicrobial nanoparticles (MPH NPs). These nanoparticles were shown to target the lungs effectively, addressing organ infections, and exhibited broad-spectrum bactericidal activity against *E. coli*, *S. aureus*, and *MRSA*, with efficacies of 100, 98.5, and 98.4%, respectively. Gold nanoparticles (AuNPs) represent an appealing delivery platform for AMPs. AMPs feature a diverse array of functional groups on their surface, including various amino acid residues. These residues facilitate the effective immobilization of peptides onto AuNPs via electrostatic interactions. Moreover, the immobilized AMPs can transition into an active conformation when appropriate, while the AuNPs

can penetrate bacterial cells to induce internal disruption and ultimately lead to bacterial death. Zhang et al. [159] coupled N-terminal cysteine-containing AMPs to AuNPs via Au–S bonds, achieving efficient assembly and conjugation. They found that the nanoparticles significantly enhanced both the antimicrobial activity and stability of the AMPs. Specifically, Au_CR (Au-Cys-Arg-NH2) exhibited selective antimicrobial activity against *S.aureus*, with a minimum inhibitory concentration (MIC) of 10 nM. Its antibacterial efficacy was comparable to or even superior to that of vancomycin and methicillin. Additionally, Au_CR induced minimal resistance and demonstrated remarkable stability, with a plasma half-life of 17.5 h. AuNPs can encapsulate AMPs through both non-covalent and covalent interactions, while metals like gold possess intrinsic antibacterial properties [160]. Consequently, the combination of AMPs and AuNPs exerts a synergistic effect in combating drug-resistant bacteria. In biomedicine, although inorganic nanoparticles are durable, their accumulation in tissues raises concerns about long-term toxicity and safety for clinical applications. Therefore, it is essential to develop metallic nanomaterials that can be rapidly cleared from the body for safer biomedical use.

In contrast, polymeric nanomaterials exhibit non-toxic, non-immunogenic, and biodegradable properties. Common natural polymers encompass chitosan (CH), collagen, while typical synthetic polymers include polycaprolactone (PCL) and polylactic acid-hydroxyacetic acid copolymer (PLGA). The encapsulation of AMPs within polymeric nanomaterials necessitates careful consideration of the nanomaterial properties, including molecular weight, hydrophobicity, charge, and dispersion index, among others [161]. PLGA, a representative polymer approved by the FDA and widely utilized, is particularly suited for preparing nanoparticles that not only serve as wound-healing agents but also sustainably release the encapsulated drug into the wound site. Kiran Kumar Chereddy et al. [162] encapsulated the antimicrobial peptide LL-37 in PLGA nanoparticles, finding that this drug delivery system significantly upregulated IL-6 and VEGF-A, enhanced angiogenesis, promoted wound healing, and achieved sustained release of LL-37 along with its intrinsic lactic acid activity. Guo et al. [163] developed poly (tannic acid)-PLGA nanoparticles, which were surface-modified with the antimicrobial peptide Dermaseptin-PP (Der), resulting in PLGA-pTA-Der nanoparticles. They discovered that NIR light-activated PLGA-pTA-Der nanoparticles exhibited remarkable bactericidal efficiency, achieving a 99% kill rate against both Gram-negative *E. coli* and *S. aureus*. Furthermore, António Miguel Ramôa et al. [164] enhanced the application of AMPs in infection therapy by conjugating them to PLGA-PEG nanoparticles (PLGA-PEG NPs). This approach not only preserved the antimicrobial activity of AMPs but also improved their efficacy. The minimum inhibitory concentrations (MICs) for Pseudomonas aeruginosa were found to be 8–16 μg/mL, and for Staphylococcus aureus, 16–32 μg/mL. Additionally, PLGA-PEG significantly accelerated the killing kinetics: for Pseudomonas aeruginosa, the time was reduced from 1–2 h to just 15 min, and for Staphylococcus aureus, it decreased from 6–8 h to 0.5–1 h. These studies have demonstrated that nanoparticles can effectively enhance the biological activity of AMPs.

Nanoparticle-targeted delivery systems leverage the binding between target-specific high-affinity ligands, enabling the precise direction of AMPs to disease sites. This approach ensures that AMPs target and eliminate bacteria directly at the infection site or are released to combat infected and activated cells. Consequently, this targeted delivery enhances AMP loading efficiency and therapeutic efficacy. Liu et al. [165] developed a nanovisualization platform (Ag@Pt-Au-LYZ/HA-LL-37 NPs) that exhibits targeted delivery to infected microenvironments and enzymes. This targeting is further enhanced by the photothermal effect, which improves the localization, retention, and antimicrobial activity of LL-37, thereby supporting precise bacterial elimination. In vitro and in vivo studies demonstrated that the minimum bactericidal concentrations of Ag@Pt-Au-LYZ/HA-LL-37 were 16 μg/mL for *S. aureus* and 32 μg/mL for *E. coli*. Moreover, when combined with near-infrared (NIR) treatment in mice, the bacterial inhibition rate reached 97% (Fig. 5).

While the use of nanomaterials in antimicrobial peptide delivery systems has significantly improved bioavailability and targeting, their clinical translation still faces numerous regulatory challenges. One key issue is the lack of precise definitions for nanomaterials or drugs. For instance, PLGA-PEG-NPs prepared by António Miguel Ramôa, which have AMPs grafted onto their surface, could be classified either as biopharmaceuticals or fall under the regulatory framework for medical devices due to the characteristics of the nanocarriers [164]. This ambiguity highlights the need for clearer guidelines to facilitate clinical translation. Secondly, the safety assessment of nanomedicines requires stringent requirements, such as higher cytotoxicity when the zeta potential is positive [166]. And uneven particle size distribution affects renal clearance [167]. At last, the issue of non-harmonization of nanoparticle standards leads to a lack of clear guidance and increased uncertainty in the process of clinical translation of nanoparticles. For example, there is a lack of international standard substances for potency determination of antimicrobial peptide nano-formulations and there is no consensus on release

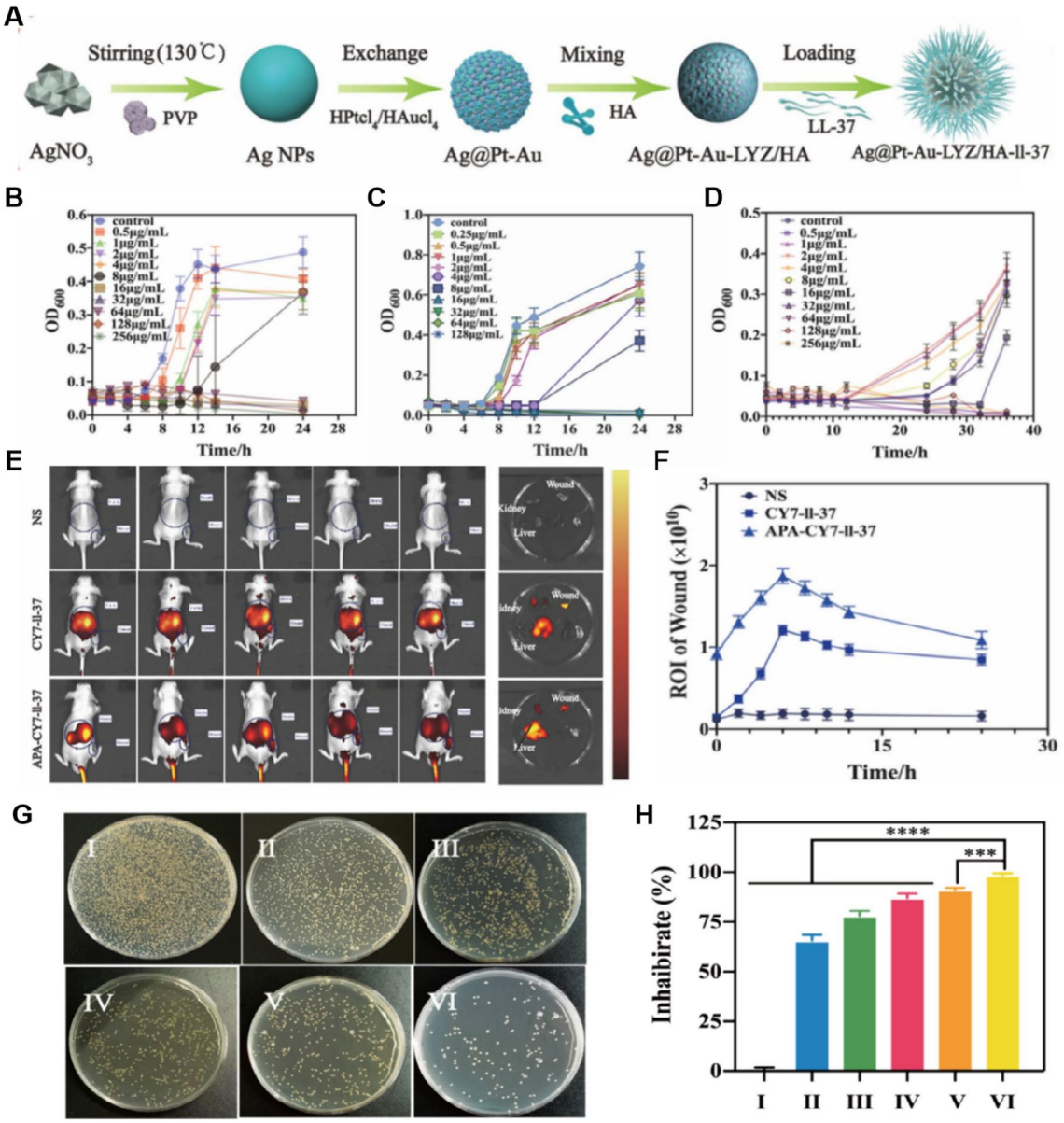


Fig. 5 **A** Schematic structure of Ag@Pt-Au-LYZ/HA-LL-37NPs and flow chart for their preparation. **B–D** Growth curves of in vitro antimicrobial effects of Ag@Pt-Au-LYZ/HA-LL-37NPs against *S. aureus*, *E. coli* and *Bacillus subtilis*. **E** In vivo fluorescence imaging images of CY7-LL-37 and Ag@Pt-Au-LYZ/HA-LL-37 at the trauma site after administration of CY7-LL-37 as well as changes in fluorescence intensity of internal organs and wounds, which are indicated by the red highlighted areas. **F** Quantitative fluorescence analysis of (**E**). **G** Images of colonies of in vivo wound homogenates cultured in agar petri dishes. **H** Assessment of bacteriostatic activity in wound tissue by different groups. The treatment groups were (I) blank control group, (II) AgNPs group, (III) Ag@Pt-Au, (IV) Ag@Pt-Au-LYZ/HA, (V) Ag@Pt-Au-LYZ/HA-LL-37, and (VI) Ag@Pt-Au-LYZ/HA-LL-37 + NIR. (from Liu et al. [165]). The image has been reproduced with permission from publisher

kinetic assays, which creates barriers to the exchange of data between global multicenter clinical trials [168]. Currently, the global regulation of nanomaterial-based medical products is still evolving. In recent years, numerous guidance documents have been published worldwide. For instance, the U.S. FDA has issued guidelines for the use of nanomaterials in medical devices and pharmaceuticals [169]. The European Union has also introduced new recommendations focusing on the physicochemical properties of nanomaterials [170].

In this context, clinical trial research involving nanomaterials has also advanced more rapidly. Currently, over 100 nanomedicines are available on the market, with approximately 600 more in various stages of clinical

development [171, 172]. These drugs primarily target the treatment of cancer and infections, and their applications have expanded to include neurological disorders, hematological conditions, metabolic diseases, and numerous other therapeutic areas. Nanoparticles have also played a crucial role in vaccine development and diagnostic imaging [173]. For instance, Sun et al [174]. utilized nanorods as substrates, modifying their surfaces with PEG and 64Cu, which enabled successful application in PET imaging. Currently, no clear public evidence indicates that AMPs nanomedicines have entered clinical trials, yet many are actively progressing from the laboratory toward clinical application. Ju et al. [175] in collaboration with the Eye Hospital of Tianjin Medical University, addressed the challenge of the corneal stromal barrier in fungal keratitis by coupling AMPs with polysaccharides. They designed peptide-conjugated nanosubstances capable of rapidly penetrating the entire corneal stroma to eliminate pathogens without causing ocular surface irritation. This AMP-based nanomedicine is now being prepared for phase I clinical trials. In conclusion, these examples highlight the significant potential of nanomaterial-based AMPs for clinical applications.

Status and challenges of clinical translation of AMPs

As outlined above, AMPs hold promising application prospects owing to their diverse biological activities, immunomodulatory properties, and the ability to effectively combat multidrug-resistant bacteria. There has been extensive laboratory research on AMPs, resulting in the development of 11,612 such peptides. However, despite significant efforts in designing and developing delivery systems for AMPs, only a limited number of AMP-based drugs are available for clinical use. Currently, just 11 bacteriophage peptide drugs are on the market, including mucin, polymyxin B, vancomycin, shortening, baclofenacin, daptomycin, and others. Table 2 provides a summary and detailed overview of the marketed antimicrobial peptide drugs [166].

Many challenges hinder the clinical translation of AMPs. Primarily, these challenges stem from the inherent properties of AMPs themselves. They are susceptible to degradation by proteases and have a short half-life. While AMPs exhibit antibacterial effects in vitro, they often fail to maintain an effective therapeutic concentration in vivo when used clinically. Consequently, AMPs cannot sustain their role at clinical target sites, especially for chronic infections where antimicrobial agents need to persist over extended periods to effectively eliminate bacteria. The inability of AMPs to maintain adequate therapeutic concentrations in the body further complicates their clinical application. Iseganan, Augmentin, Pexigarnan, Sulomycin, Neuprex, and XMP-629 all advanced to Phase III trials. However, their therapeutic concentrations either reduced efficacy or failed to demonstrate significant superiority over existing antibiotics, which prevented these drugs from successfully completing the Phase III clinical trial process [167]. Moreover, the complex mechanisms of action of AMPs, along with the cytotoxicity and immunogenicity of certain AMPs, significantly impact the evaluation of their safety and dosing in clinical translation. These factors limit the application scope and dosage of AMPs [168]. Zhibo Gai et al. [169] discovered that mucomycin can cause a certain degree of nephrotoxicity, which occurs relatively quickly and at a high incidence, necessitating careful adjustment of the dosage. Gramicidin exhibits severe cytotoxicity, restricting its use to topical ointments for treating skin infections [170]. Another disadvantage of AMPs is the challenge in achieving large-scale industrial production due to expensive raw materials, complex preparation processes, and low yields. Currently, the main preparation methods for AMPs include chemical synthesis, genetic engineering, and biological extraction. While chemically synthesized AMPs offer precise control over peptide length and high purity, this method is costly, especially for longer chains, and amino acids are prone to deconvolution. Genetically engineered AMPs may reduce costs but often suffer from low activity or expression levels, which can drive up expenses [171]. Additionally, biological extraction methods are complicated and not conducive to large-scale production [172]. These factors collectively pose significant challenges for the industrialization of AMPs. At the same time, even if the production of AMPs is achieved, their drug prices would likely remain high. This makes antimicrobial peptide drugs less competitive in the market and unable to meet the clinical demand for affordable, high-volume antimicrobial medications [171]. Various factors inherent to AMPs, as well as challenges related to industrial-scale production, have hindered their clinical application.

Despite the shortcomings of AMPs and the challenges in industrialization, researchers remain highly interested in their development and clinical translation. The clinical translation of AMPs must consider not only safety and efficacy but also patient compliance. Most AMPs are currently restricted to topical or intravenous administration. To enhance patient compliance, oral formulations such as vancomycin hydrochloride capsules have been developed [173]. However, these oral alternatives exhibit reduced efficacy and a narrower therapeutic range compared to intravenous administration, limiting their widespread adoption. Presently, several oral AMPs are undergoing clinical trials, including NVB-302, sulforaphane, and remeronin. These clinical studies hold significant

Table 2 Drugs for AMPs already on the market

Name	Description of antimicrobial peptides	Clinical applications of antimicrobial peptides	Antimicrobial peptide drug originator	First time on the market
Daptomycin	Daptomycin is a cyclic lipopeptide antibiotic used to treat complicated skin and skin structure infections by susceptible Gram-positive bacteria and bacteremia due to Staphylococcus aureus	Treatment of complicated skin and skin structure infections (cSSSI), Staphylococcus aureus bloodstream infections (bacteremia)	Cubist Pharmaceuticals LLC (Merck & Co.)	September 2003
Dalbavancin	Dalbavancin is a semisynthetic lipoglycopeptide and derivate of teicoplanin. It is an antibacterial used to treat acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible strains of Gram-positive bacteria	Acute bacterial skin infections, Osteomyelitis and septic arthritis	Actavis plc	May 2014
Telavancin	Telavancin is a semi-synthetic derivative of vanocymycin that has bactericidal activity against Methicillin-resistant Staphylococcus aureus (MRSA) and other gram-positive bacteria	Osteomyelitis, Bacterial infections	Clinigen Group plc/Innoviva Inc./Pendopharm/Theravance Biopharma Inc./University of Illinois	September 2009
Oritavancin	Oritavancin is a glycopeptide antibiotic used to treat acute bacterial skin and skin structure infections caused by susceptible Gram-positive bacteria	Treatment of adult patients with acute bacterial skin and skin structure (including subcutaneous) infection	The Medicines Company	August 2014
Bacitracin	Bacitracin A is a homodetic cyclic peptide consisting of (4R)-2-[(1S,2S)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid attached head-to-tail to L-leucyl, D-glutamyl, L-lysyl, D-ornityl, L-isoleucyl, D-phenylalanyl, L-histidyl. D-aspartyl and L -asparaginyl residues coupled in sequence and cyclised by condensation of the side-chain amino group of the L-lysyl residue with the C-terminal carboxylic acid group. It is the major component of bacitracin. It is a homodetic cyclic peptide and a polypeptide	prevent wound infections, treat pneumonia and empyema in infants, and to treat skin and eye infections	Pfizer	January 1945
Polymyxin-E	Polymyxin-E is a pcaused by susceptible Gram negative bacteria. olymyxin antibiotic used to treat bacterial infections	Treatment of acute or chronic infections due to sensitive strains of certain gram-negative bacilli, particularly Pseudomonas aeruginosa	ParSterile Products	June 1970
Polymyxin B	Polymyxin B is a polymyxin antibiotic used to treat a wide variety of infections in the body.They are basic polypeptides of about eight amino acids and have cationic detergent action on cell membranes	Treatment of infections of the urinary tract, meninges, and blood stream, caused by susceptible strains of Pseudomonas aeruginosa	Glaxo Wellcome UK Ltd	December 1952
Tyrothricin	A polypeptide antibiotic mixture obtained from Bacillus brevis. It consists of a mixture of three tyrocidines (60%) and several gramicidins (20%) and is very toxic to blood, liver, kidneys, meninges, and the olfactory apparatus. It is used topically	Treatment of infected skin and infected oropharyngeal mucous membranes	René Dubos	1942

Table 2 (continued)

Name	Description of antimicrobial peptides	Clinical applications of antimicrobial peptides	Antimicrobial peptide drug originator	First time on the market
Vancomycin	Vancomycin is a glycopeptide antibiotic used to treat severe but susceptible bacterial infections such as MRSA (methicillin-resistant Staphylococcus aureus) infections	Treatment of septicemia, infective endocarditis, skin and skin structure infections, bone infections, and lower respiratory tract infections	Eli Lilly & Co	January 1954
Gramicidin S	Cyclic peptide biosynthesized from gramicidin in Bacillus brevis; comprises two identical pentapeptides coupled head to tail	Potent against gram-negative and gram-positive bacteria and fungi restricted use as spermicide and to treat genital ulcers caused by STD	/	/
Gramicidin D	Gramcidin D is a heterogeneous mixture of three antibiotic compounds, gramicidins A, B and C, making up 80, 6, and 14% respectively all of which are obtained from the soil bacterial species Bacillus brevis and called collectively gramicidin D. Gramcidins are 15 residue peptides with alternating D and L amino acids, which assemble inside of the hydrophobic interior of the cellular lipid bilayer to form a β-helix	Skin lesions, surface wounds and eye infections	/	/

Data from: Antimicrobial Peptide Database DRAMP, Drug Clinical Trial Registration and Information Publication Platform, WHO International Clinical Trial Registry Platform

promise for advancing the development of effective oral AMP therapies [167].

With the advancing development of AMPs, nearly 50 AMP-based drugs have entered clinical trials, with 14 progressing to Phase III trials (as shown in Table 3). Owing to their diverse biological activities, AMPs hold potential not only for treating infectious diseases but also for addressing other conditions. Consequently, half of the AMPs in Phase III clinical trials are being evaluated for non-infectious diseases, including diabetes, cancer, and acromegaly. Moreover, the remaining AMPs in clinical Phases I and II have also demonstrated the superior therapeutic efficacy of AMPs. For instance, the specific antimicrobial peptide (STAMP) C16G2 was used to target cariogenic oral pathogens, such as Streptococcus mutants, and successfully completed a Phase II clinical trial in 2022 [174]. Ramoplanin (NTI-851), an antimicrobial peptide produced by Actinoplanes species, completed a Phase II clinical trial for the oral treatment of vancomycin-resistant Enterococcus species and is currently in Phase II trials for treating Clostridium difficile [175].

Comparison between AMP and antibiotics

Although AMPs have shown therapeutic potential across various disease areas in clinical trials, and many are currently undergoing clinical trial, their clinical conversion rate remains significantly lower than that of conventional antibiotics. This disparity arises from the systematic differences between these two classes of antimicrobials in terms of their mechanisms of action, clinical application characteristics, and industrialization maturity (Tables 2, 3). A detailed analysis of these fundamental differences will provide a critical scientific foundation for optimizing anti-infective treatment strategies.

From a mechanistic perspective, antibiotics predominantly operate through a "key-lock" single-target mode of action. For instance, β-lactams inhibit penicillin-binding proteins to achieve their antimicrobial effects [176]. However, this mechanism is vulnerable to failure due to target mutations. Clinical monitoring data reveal that bacterial resistance rates are alarmingly high: 51% for penicillin, and the median resistance rate for third-generation cephalosporins in *E. coli* is 36%. Furthermore, resistance to ciprofloxacin in *E. coli* varies from 8.4% to a concerning 92.9%, while in *K. pneumoniae*, it ranges from 4.1 to 79.4% [177]. In contrast, AMPs can have multiple targets or multiple AMPs can act on the same target, thereby effectively minimizing the emergence of bacterial resistance [178]. However, the industrialization of AMPs is challenged by significantly high production costs, estimated at US$50–400 per gram of amino acids for commercial-scale production [167]. In contrast, traditional antibiotics like penicillin G can be produced at a much lower cost of approximately $10 per kilogram using well-established fermentation processes [179]. While the high production cost of AMPs leads to higher selling prices, the shorter treatment duration can result in reduced hospitalization and overall medical expenses.

The differences between the two are further emphasized by the differentiation of clinical application scenarios. The low metabolic stability and oral bioavailability of AMPs, coupled with short half-life limitations, have led to the most common routes of administration for AMPs being topical, including cream and emollient administration, wound or surgical site administration, and mucosal application as a nasal spray [180]. However, antibiotics are still used as the main drug in the treatment of critical illnesses such as some bloodstream infections, which is also mainly related to the fact that the systemic toxicity of AMPs has not yet been clarified [181]. It is worth noting that the regulatory system of AMPs is undergoing changes. China will release the National Action Plan for Containing Microbial Resistance (2022–2025) in 2022, which will prioritize the review and approval of new drugs, vaccines, and innovative medical devices that are urgently needed for the prevention, diagnosis, and treatment of drug-resistant infections in accordance with procedures. In addition, at the 77th World Health Assembly, WHO proposed the topic "Antimicrobial Resistance: Accelerating National and Global Responses", in which WHO supports innovative initiatives that contribute to the research and development of alternatives to traditional antibiotics in the hope of solving the problem of antimicrobial resistance [182]. All of these changes have accelerated the development of AMPs, such as speeding up the clinical trial process for pecelegan spray in a variety of open wound infections caused by bacteria, including diabetic foot, decubitus ulcers, burns, etc. [183]. This has led to the drug's 2024 marketing application being accepted by China's State Drug Administration Drug Review Center. This also demonstrates that AMPs are developing into a core component of precision anti-infective therapy, especially in localized high bacterial load and biofilm-associated infection scenarios highlighting the clinical value (Table 4).

Summary and discussion

Antimicrobial peptides, a class of biologically active molecules with unique antimicrobial mechanisms, demonstrate significant potential in biomedical applications. These peptides target cell membranes, cell walls, and multiple intracellular sites, reducing the likelihood of bacterial drug resistance and positioning themselves as ideal alternatives to address the current antibiotic resistance crisis. Beyond their antibacterial effects, AMPs

Table 3 Drugs for AMPs in clinical phase III

Name	Description	Application	R&D Company
p2TA(AB103, Reltecimod)	Reltecimod is under investigation in clinical trial NCT02469857 (Phase III Efficacy and Safety Study of AB103 in the Treatment of Patients With Necrotizing Soft Tissue Infections)	Necrotizing soft tissue infections	Atox Bio Ltd
Ramoplanin(NTI-851)	Ramoplanin (NTI-851) is a macrocyclic glycolipodepsi peptide produced by Actinoplanes spp. being developed by Nano-therapeutics. It exhibits bactericidal activity by blocking the cell wall peptidoglycan synthesis of gram-positive bacteria. Recently, the phase III clinical study of the peptide was initiated for the oral treatment of vancomycin-resistantenterococcus (VRE) colonization, as well as the phase II trial against Clostridium difficile	Treatment of bacterial infections	Nano-therapeutics
XMP 629(HY-P2170; CS-0109623)	Extracted from human	Impetigo and acne rosacea	Xoma Ltd(Berkeley, CA, USA)
Mycoprex	Extracted from insects	Fungal infections	Xoma Ltd
Talactoferrin alpha (Lactoferroxin-A)	Talactoferrin alfa is a novel immunomodulatory 80 kD protein with demonstrated oral anti-tumor properties. Lactoferrin, a protein found in breast milk is developed by Agennix. It increases body's immune power and also works as a natural antioxidant, helping to control cell and tissue damage caused by oxidation	Combined with chemotherapy in the first-line treatment of advanced non-small cell lung cancer; Topical treatment in diabetic neutropathic ulcers	Agennix
D2A21 (Demegal, Provena)	A 22-residue αhelix peptide	Burn infection, skin infection with multidrug-resistant pathogens	Demegen
Glutoxim (NOV-002)	Glutathione Disulfide NOV-002 is a stabilized formulation of disodium glutathione disulfide (GSSG; oxidized glutathione) and cisplatin (1000:1) with potential chemoprotective and immunomodulating activities	Tuberculosis, non small cell, lung cancer	Pharma BAM/Novelos
XOMA-629	9-amino-acid peptide derivative of bactericidal/permeability-increasing protein	Impetigo	Xoma
DiaPep277	DiaPep277 is a small, lyophilized powder containing 24 Amino-acids. It has proved in former studies that DiaPep277 can slow down beta cells destruction in the pancreas and therefore decelerate the progress of Diabetes	Type 1 diabetes mellitus	DeveloGen
Dusquetide(SGX942)	Dusquetide is a synthetic, 5-amino acid peptide and Innate Defense Regulator (IDR), with immunomodulating, anti-inflammatory, anti-infective and anti-mucositis activities	Treatment for oral complications caused by radiation therapy for head and neck cancer	Soligenix

Table 3 (continued)

Name	Description	Application	R&D Company
Omiganan (MBI-226/MX-226/CLS001)	Omiganan (MBI-226), an analogue of indolicidin, has been proven to be capable of significantly reducing catheter colonization and microbiologically confirmed tunnel infections during catheterization	Rosacea, Acne vulgaris (II), Genital warts (II)	Maruho Co., Ltd(developing), Cutanea Life Sciences, Inc. (developing), Mallinckrodt, Micrologix Biotech(Vancouver, BC, Canada)
PL-5	This product is a national category 1 anti-infective innovation category in China, and has obtained PCT US and Chinese invention patent authorizations	Skin wound infection	Changchun ProteLight Pharmaceutical & Biotechnology Co
Surotomycin(MK-4261/CB-183,315)	Surotomycin has been used in trials studying the treatment of Diarrhea and Clostridium Difficile Infection. It is a benzenebutanoic acid derivative patented by Cubist Pharmaceuticals, Inc. as antibacterial agents for the treatment of Gram-positive infections	Treatment of Diarrhea and Clostridium Difficile Infection	Cubist Pharmaceuticals Inc./Merck & Co. Inc
TD-1792(Cefilavancin)	Cefilavancin is a covalently-linked glycopeptide-cephalosporin (beta-lactam) heterodimer antibiotic that exhibits substantially greater activity than its component parts against Gram-positive bacteria	Gram-positive infections, Skin and soft tissue infections	GlaxoSmithKline Co., Theravance Biopharma Inc., R-Pharm

Data from: Antimicrobial Peptide Database DRAMP, Drug Clinical Trial Registration and Information Publication Platform, WHO International Clinical Trial Registry Platform

Table 4 Comparison of antibiotics and antimicrobial peptides

	Antibiotics	Antimicrobial peptide
Mechanism of action	Single target (e.g., enzyme inhibition, cell wall synthesis blockade)	Multi-target (membrane cleavage, nucleic acid binding, immunomodulation)
Risk of drug resistance	High (target mutation or inactivation enzyme generation)	Low (multi-target synergy, high target conservation)
Antimicrobial spectrum	Narrower (strain-specific)	Broad spectrum (effective against bacteria, fungi, viruses)
Immunomodulatory function	No	Yes
Production costs	Low (mature chemical synthesis process)	High (higher cost of peptide chain synthesis or gene expression)
Clinical applicability	Widespread (systemic infection)	Restricted (localized infections predominate, carrier system required for systemic delivery)
Toxicological risk	Low to moderate (some antibiotics have liver and kidney toxicity)	Higher (hemolysis, cytotoxicity to be optimized by modification or delivery system)
Combination therapy potential	Susceptible to antagonistic effects	Enhance antibiotic penetration

exhibit a broad spectrum of biological activities, including anti-inflammatory and antiviral properties. However, despite their promise, these peptides face certain limitations such as susceptibility to protease degradation in vivo, poor stability, and a short half-life, which constrain their clinical application.

To overcome these shortcomings, various drug delivery systems have been developed. AMPs can be chemically modified (e.g., cyclization, acetylation) or integrated into delivery vehicles (nanoparticles, liposomes, hydrogels) to significantly improve their stability against proteases, reduce cytotoxicity, and precisely regulate half-life and release kinetics. These systems lay the technological foundation for clinical translation by improving the pharmacokinetic and pharmacodynamic properties of AMPs, increasing stability and bioavailability, reducing off-target effects and toxicity, and modulating efficacy in the local microenvironment. However, future research needs to focus on the following key issues: (1) designing an intelligent delivery system to achieve infection site-specific drug release and avoid systemic infection. (2) Explore drug delivery strategies (e.g., antibiotic-antimicrobial peptide co-delivery system) to delay the development of drug resistance through synergistic effects. (3) Establish a standardized evaluation system for antimicrobial peptide drug delivery systems.

The advantages of AMPs and advancements in drug delivery systems aimed at overcoming some of their limitations, clinical translation continues to face numerous challenges. Currently, there are 11,612 known AMPs, with approximately 50 in clinical trials, and only 11 having reached the market. These challenges stem not only from inherent limitations of AMPs but also from various issues in their industrial production. Key obstacles include the high costs and complex processes associated with large-scale manufacturing, which hinder meeting substantial clinical demands. Additionally, there is insufficient research on the long-term safety and efficacy of AMPs in vivo, leading to slower progress in clinical trials.

With the rapid advancement of science and technology, numerous key technologies have emerged that significantly facilitate the clinical translation of AMPs. In AMPs design, artificial intelligence can predict and optimize peptide structures with greater precision. Regarding production technology, optimizing genetic engineering techniques substantially boosts the yield and reduces the cost of AMPs. For instance, utilizing high-efficiency expression vectors and host cells enables large-scale production of AMPs. Meanwhile, advancements in materials science offer the potential to design more efficient and safer drug delivery systems. For instance, smart-responsive carriers can precisely release AMPs in specific physiological or pathological environments. Furthermore, high-throughput screening technology facilitates the rapid identification of antimicrobial peptide lead compounds that exhibit high activity and low toxicity, thereby accelerating the drug development process.

In this study, we believe that with the advancement of these technologies, the clinical development of AMPs will primarily focus on three directions: precision therapy, co-administration strategies, and multifunctional formulations. (1) As understanding of the mechanisms of action of AMPs deepens, they are becoming precision therapeutic agents for treating specific diseases. (2) Combining AMPs with antibiotics, immunomodulators, and other drugs can produce synergistic effects, thereby maximizing the therapeutic index while minimizing the development of drug resistance and other adverse reactions. (3) By integrating multiple disciplines, we can develop multifunctional drug delivery systems. For instance, combining AMPs with nanotechnology and biosensing technology could yield intelligent

nano-formulations capable of real-time monitoring of pathogen infections and precise treatment. Additionally, composite formulations with multiple functions, such as antimicrobial activity and promotion of tissue repair, can be developed.

In conclusion, this paper offers a comprehensive and in-depth review of the biological activities and delivery systems of AMPs. It provides a detailed analysis of AMP drugs currently in clinical trials as well as those successfully applied clinically, and examines the critical bottlenecks in their current clinical translation process. This review will provide a clear idea for the design of novel AMPs and the development of new drug delivery systems, and provide practical solutions to promote the translation of AMPs in clinical applications and help AMPs make new breakthroughs in the field of medicine.

Author contributions
S.N. and S.L. contributed to the conception of this review. S.N., S.L.and C.F. analyzed literatures and wrote the manuscript. Y.H., G.E. and L.B. completed figures drawing. S.N., L.B., S.L. and Y.H. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Declarations

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Received: 21 January 2025 Accepted: 25 February 2025
Published online: 07 March 2025

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