Skin and wound delivery systems for antimicrobial peptides

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Skin and wound delivery systems for antimicrobial peptides
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Abstract
Non-healing wounds cause hundreds of thousands of deaths every year, and result in large costs for society. A key reason for this is the prevalence of challenging bacterial infections, which may dramatically hinder wound healing. With resistance development among bacteria against antibiotics, this situation has deteriorated during the last couple of decades, pointing to an urgent need for new wound treatments. In particular, this applies to wound dressings able to combat bacterial infection locally in wounds and impaired skin, including those formed by bacteria resistant to conventional antibiotics. Within this context, antimicrobial peptides (AMPs) are currently receiving intense interest. AMPs are amphiphilic peptides, frequently net positively charged, and with a sizable fraction of hydrophobic amino acids. Through destabilization of bacterial membranes, neutralization of inflammatory lipopolysaccharides, and other mechanisms, AMPs can be designed for potent antimicrobial effects, also against antibiotics-resistant strains, and to provide immunomodulatory effects while simultaneously displaying low toxicity. While considerable attention has been placed on AMP optimization and clarification of their mode(s)-of-action, much less attention has been paid on efficient AMP delivery. Considering that AMPs are large molecules, net positively charged, amphiphilic, and susceptible to infection-mediated proteolytic degradation, efficient in vivo delivery of such peptides is, however, challenging and delivery systems needed for the realization of AMP-based therapeutics. In the present work, recent developments regarding AMP delivery systems for treatment of wounds and skin infections are discussed, with the aim to link results from physicochemical studies on, e.g., peptide loading/release, membrane interactions, and self-assembly, with those on the biological functional performance of AMP delivery systems in terms of antimicrobial effects, cell toxicity, inflammation, and wound healing.

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Introduction
Through its multi-layer structure (and particularly its stratum corneum layer) skin provides a formidable barrier, which not only controls water and electrolyte balances of the body, but also prevents pathogens in the environment from entering into the body. Disruption of the skin tissue results in wound formation. Normally, wounds heal through a sequence of processes, i.e., inflammation, proliferation, hemeostasis, and remodelling. Although the surface of skin is richly colonized by bacteria, most wounds heal without problem. Sometime, however, the healing process may be challenged by the presence of pathogenic bacteria, and effects of these aggravated by co-factors such as devitalized tissue or inflammation. This, in turn, may result in non-healing wounds, including diabetic, venous, and pressure ulcers [1].

Non-healing wounds contribute to massive suffering and to considerable socioeconomic costs. For example, burn wounds results in about 180,000 deaths per year. In USA, the treatment cost is $10.4 billion per year, with an average cost of more than $1,600,000 to treat severe burns in the absence of any complications [2]. Analogously, infected wounds is a frequent cause of death in diabetic and immunocompromised patients. With the development of multi-drug resistance (MDR) in bacteria, the situation is on its way to be further aggravated. Being non-responsive to antibiotics, MDR bacteria severely hinder wound healing. The so-called ESKAPE bacteria (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species) are particularly bad in this respect. In addition, S. aureus and P. aeruginosa may form biofilms, which worsens the situation still since bacteria in biofilms are 100-1000-fold more tolerant to antimicrobial agents [3].

Current treatments of infected wounds are based on dressings (e.g., hydrogels or fiber mats) able to absorb...
liquid and provide a moist environment to optimize skin repair and protect against infections. Iodine is commonly used (both alone and in dressings) as a broad-spectrum antimicrobial compound. Another frequently used antimicrobial agent is silver, including silver salts and colloidal silver. In addition, various antibiotics are employed, including polymyxin B, nystatin, bacitracin, mafenide acetate, and neosporin. Other components in skin dressing include biodegradable polymers (e.g., hyaluronic acid) to promote healing and chitosan to stop bleeding [2]. For more severe cases, infected wounds may lead to sepsis, with has a staggering 20—40% mortality rate [4]. For such cases, additional treatment regiments include fluid resuscitation and intravenous antibiotics but these are often inadequate due to compromised vasculature limiting the effectiveness of systemic antibiotics [5].

Considering the rapid development among bacteria against conventional antibiotics and other antimicrobial agents, as well as the major suffering and socioeconomic costs related to wound and skin infection, there is thus an urgent need for new types of antimicrobial skin dressings for wounds and impaired skin. Here, antimicrobial peptides (AMPs) provide an interesting opportunity. Such peptides may provide potent antimicrobial and anti-inflammatory effects, yet displaying low toxicity [6,7]. The different mechanisms contributing to such effects continue to be intensively investigated, and peptides continuously optimized from perspectives of potency and selectivity. Much less attention has, however, been paid to delivery systems for AMPs, despite these peptides being large, cationic, and amphiphilic, hence in need of delivery systems [8]. In the present summary, we therefore describe recent developments regarding AMP delivery systems to combat skin and wound infections.

**Nanogels, microgels, and hydrogels**

Nanogels and microgels are particle variants of macroscopic hydrogel networks, the degree of swelling of which may be controlled by ambient conditions and external stimuli. Properties of nanogels, microgels, and hydrogels making such systems interesting as AMP delivery systems for skin and wound infections include frequently favourable biocompatibility, good colloidal stability, high water content, and a network structure allowing incorporation of AMPs. Investigating how AMP loading and release in microgels influence antimicrobial effects and cell toxicity, Nordström et al. reported anionic acrylic acid-based microgels to incorporate large amounts of the cationic AMPs DPK-060 (GKHK NKGKKNGKHNGWKWWW) and LL-37 (LLGDFF RKSKEKIGEKFIRVQRIKDFLRNLVPRTES), stabilizing these from infection-related proteolytic degradation. Due to their negative charge, these microgels did not adsorb at bacteria-like membranes. Instead, lysis of such membranes was found to require peptide release. Mirroring this, antimicrobial effects against methicillin-resistant *S. aureus* (MRSA), *Escherichia coli* (*E. coli*) and *P. aeruginosa* were found to require peptide release. Microgels were also demonstrated to trigger low hemolytic effects, and suppress such effects, compared to free peptide, in the case of LL-37 [9] (Figure 1).

Nystrom et al. reported on the same type of microgels loaded with KYE28 (KYEITTHNLFRLTHRFR NFGYTLR), as well as its PEGylated version, KYE28PEG. The results showed that peptide binding to the microgels was dominated by electrostatics, thus higher at higher negative microgel charge, while PEGylation suppressed peptide uptake into the microgels. Due to the dominating role of electrostatics, peptide release was facilitated at high (physiological) ionic strength, notably so for KYE28PEG. Furthermore, microgel-modified surfaces displayed potent suppression of *E. coli* binding, irrespective of peptide loading. For peptide-loaded microgels, contact killing provided the primary antimicrobial effect at low ionic strength, whereas at high ionic strength, released peptides contributed increasingly to antimicrobial effects. In addition, KYE28- and KYE28PEG-loaded microgels displayed anti-inflammatory effects on human monocytes, mirroring that of the free peptides [10]. In yet another study of this type, Nordström et al. investigated degradable anionic dendritic nanogels (DNG) consisting of carboxylic acid-based anionic binding sites for cationic AMPs, surrounded by PEG chains, able to load both DPK-060 and LL-37. While these nanogels were biodegradable, peptide release was much faster than nanogel degradation, and bacterial membrane destabilization largely independent of the nanogel degradation [11].

Investigating the performance of nanogel-loaded AMPs, Sun et al. investigated incorporation of the peptide OH-CATH30 (OH30; KFFKGLKNSVKKRAKFFKPR-VIGVSPF) into carboxymethyl chitosan nanogels. Peptide release from such particles was found to be maintained for at least 24 h. In full-thickness wounds in KM mice, the peptide-loaded particles displayed improved wound healing compared to peptide or polymer alone. Histopathological examination suggested that wound healing was promoted by enhanced granulation tissue formation. Furthermore, a steady expression of anti-inflammatory IL10 was observed, together with down-regulated cytokine expression, illustrating the anti-inflammatory properties of the formulation [12].

Obouo et al. reported on a hydrogel system consisting of cross-linked DNA, to which the AMP L12 was incorporated. Peptide release from the resulting hydrogels was gradual and could be controlled by DNase concentration. Loading of L12 into DNA hydrogels suppressed peptide toxicity, and loaded hydrogels...
displayed modest cell toxicity against mammalian dermal keratinocytes, but still antimicrobial activity against \textit{in vitro}. In a porcine infection model, the peptide-loaded hydrogel resulted in potent effects after 24 h. Moreover, the DNA-based hydrogels were found to display a strong anti-inflammatory response. As a result of that, faster healing was observed in mice excision wounds [13] (Figure 2). Furthermore, Wei et al. reported on a hyaluronic-based hydrogels, able to suppress inflammation (through incorporation of the AMP SWLSKAKKLFFKKIPPKKRPRPWPPRPNM-I-NH\textsubscript{2}) and to promote angiogenesis and collagen deposition (through incorporation of platelet-rich plasma (PRP)). The resulting hydrogel displayed antimicrobial effects against \textit{S. aureus}, \textit{E. coli}, and \textit{P. aeruginosa}, facilitated fibroblast proliferation, and improved wound healing in mice [14].

Somewhat related, Li et al. reported on biodegradable poly(t-lactic acid)-based systems able to undergo thermosensitive \textit{in situ} gel formation, as well as its loading with the antimicrobial peptide 57 (AP-57), and the application of such systems for wound healing. AP-57 was found to be released over an extended period and exhibited low cytotoxicity against HEK 293 cells, as well as high antioxidant activity \textit{in vitro}. At room temperature, the system was liquid and could easily be applied to the wound, whereafter it solidified \textit{in situ}, thus providing an interesting formulation application procedure. Healing of full-thickness wounds in SD rats was found to be significantly improved for the peptide-loaded hydrogel [15].

Related to nanogels loading, AMPs can also be mixed with non-crosslinked polyelectrolytes to form nanoparticles. Although attractive electrostatic interactions between AMPs and oppositely charged polyelectrolytes frequently result in macroscopic phase separation, the aggregation may be tuned by various parameters, such as polyelectrolyte and peptide charge, mixing ratio, and ambient conditions (e.g., ionic strength and pH). Together, these can be used to finely tune the aggregation, allowing precise control of the structure, composition, and structure of the nanoparticles formed.
Schematic illustration of DNA-based hydrogels as AMP delivery systems. Peptide release from the resulting hydrogels was gradual and could be controlled by DNAse concentration. Loading of L12 into DNA hydrogels suppressed peptide toxicity, and loaded hydrogels displayed modest cell toxicity against mammalian dermal keratinocytes, but still antimicrobial activity in vitro (d). In vivo, L12-loaded DNA hydrogels revealed potent antimicrobial effect on porcine explant infections (e) (Redrawn from Ref. [13]).
Illustrating the use of such systems for AMP delivery, Insua et al. reported on nanoparticles formed by cationic polymyxin B and anionic poly(styrene sulfonate) (PSS). The antimicrobial effects of the nanoparticles against *P. aeruginosa* expectedly increased with increasing polymyxin B concentration, but also depended on formulation composition. In particular, higher Mw PSS resulted in more stable nanoparticles, which suppressed peptide release and antimicrobial effect. By tuning the polyelectrolyte Mw and peptide/polyelectrolyte mixing ratio, the antimicrobial effect against *P. aeruginosa* could be improved by a factor of $10^4$ [16]. Also reporting on the functional performance of AMP-polyelectrolyte complexes, Wang et al. investigated nanoparticles formed between MSI-78 (GIKFKLLKKFGKAFVKILKK-NH$_2$) and methoxy poly(ethylene glycol)-b-poly(α-glutamic acid). Expectedly, MSI-78 incorporation increased with increasing polymer concentration. Functionally, incorporation of MSI-78 into polyelectrolyte complexes was found to suppress peptide toxicity at maintained antimicrobial activity [17].

**Electrospun polymer fibers**

In electrospinning, one or several polymer solutions are injected through a metal syringe under an applied voltage. Through this, droplets are elongated until the surface tension is overcome, and a liquid jet is ejected. As a result of solvent evaporation, the jet gradually solidifies into a fibre before reaching the collector stage. Depending on collector set-up, the fibers may be collected in various geometries, including, e.g., unaligned meshes or oriented assemblies. Furthermore, by using two polymer solutions and separate but converging injection sites, core-shell fibers can be obtained in coaxial electrospinning. This allows, e.g., polymers of different hydrophobicity to be used in the shell and the core of the fibers, or several different drugs or other components to be included in the different compartments of the core-shell fibers. Considering this, but also that electrospun fiber mats offer other potential advantages in wound dressings, such as good exudate absorption, good oxygen permeation, and facilitation of epithelial cell growth, such fiber mats have attracted considerable attention as would dressings [18]. Most of this interest has, however, focused on low-Mw drugs, whereas considerably less attention has been placed on AMPs [19]. One reason for this is that hydrophobic polymers (e.g., poly(caprolactone), PCL) are regularly used in electrospinning to reduce water uptake and resulting fiber mat disintegration. Due to the charged nature of AMPs, such peptides are generally poorly soluble in the solvent used to dissolve the hydrophobic polymers, as well as in the hydrophobic polymers themselves after drying. As a result of this, loading AMPs into hydrophobic polymer fibers have not been very successful. If PCL is electrospun with another (hydrophilic) polymer, however, possibilities to incorporate AMPs into the resulting fibers are improved. For example, poly(ethylene oxide) (PEO) or poly(vinyl alcohol) (PVA) both allow electrospinning in polar solvents, compatible with AMPs, and also promote swelling and peptide dissolution on rehydration. As an additional advantage, both these polymers display advantageous biocompatibility and have therefore attracted interest in electrospinning of wound dressings, including those loaded with AMPs [19].

For example, Sebe et al. incorporated APO monomer (Chex-RPDKPRPYLPFRPRPPVR-NH$_2$) into PVA nanofibers, which were subsequently cross-linked. The resulting dressings were investigated in an animal model, where grazed skin wounds in mice were infected with a nearly lethal dose of MDR *Acinetobacter baumannii* (A. baumannii) The peptide-loaded nanofibers were found to result in suppressed bacterial count and accelerated wound closure. Importantly, the antimicrobial potency of the peptide-loaded nanofibers was higher than that of intramuscularly administrated peptide using only one tenth of the peptide dose [20]. Furthermore, Song et al. reported on silk fibroin nanofibers loaded with Cys-KR12 (KRIVQRKDFLR). The peptide-loaded nanofibers displayed potent antimicrobial activity against pathogenic *S. aureus*, *P. aeruginosa*, and *E. coli*, but still allowed growth and differentiation of fibroblasts and keratinocytes. In addition, immunomodulatory effect of nanofiber-loaded Cys-KR12 were observed in murine monocytes cells as a significant suppression in the expression of the cytokine TNF-α [21].

Similarly, Teixeira et al. reported on a fiber mat, formed by electrospinning of mixtures of cellulose acetate (CA) and PVA, followed by glutaraldehyde cross-linking. The resulting fibers had a diameter of $\approx 200$–$300$ nm, were uniform in structure, and displayed good miscibility between the two polymers. CA incorporation resulted in enhanced swelling, as well as in reduced water vapor and air permeability, thus preventing wound drying. After loading of cys-pexiganan (CGIGKFLKKAKKFGKAFV-KILKK-NH$_2$) and Tiger 17 (e-WCKPPKPRCH-NH$_2$) to the surface of the fiber mats, time-kill results demonstrated good antimicrobial effects against *P. aeruginosa* and *S. aureus*, as well as modest toxicity against L929 fibroblasts and HaCaT keratinocytes [22]. Along the same line, Su et al. reported on the delivery of 17BIPHE2 (GBKRLVQRLKDBLRNLV) by electrospun Pluronic F127/PCL core–shell nanofibers. After an initial burst, sustained release of 17BIPHE2 was observed over 4 weeks. The loaded nanofibers displayed potent antimicrobial effects against MRSA, *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical strains at simultaneously low toxicity to monocytes and skin cells. Furthermore, a >5-log decrease of was observed in a type II diabetic mice wound model. When combined with debridement, the peptide-loaded nanofibers were found to completely eliminate biofilms formed by both *P. aeruginosa* and MRSA [23] (Figure 3).
Peptide self-assembly systems

Due to structural and compositional versatility, as well as to functional advantages, the self-assembly of small oligopeptides has been widely researched as potential biomaterials and drug delivery systems [24]. More recently, self-assembly of larger peptides, including AMPs and related peptides have also been found to be interesting as antimicrobial biomaterials [25,26]. For example, Veiga et al. reported on self-assemblies formed by \( \beta \)-hairpin peptides, which were found to be efficient in suppressing both Gram-negative and Gram-positive bacteria, including MDR ones. The presence of arginine residues in the peptides was found to be key for this, as arginine-to-lysine substitutions suppressed antimicrobial activity [27]. Analogously, Salick et al. compared MAX1 (VKVKVKVVK(dV)PPTKVKVKVKV-NH\(_2\)) and MARG1 (VKVKVRVK(dV)PPTKVKVRVKV-NH\(_2\)) from this perspective, and found self-assemblies from both peptides to be efficient against methicillin-susceptible \( S. aureus \) (MSSA), whereas only MARG1 was efficient against MRSA, particularly at high bacterial load [28].

Reporting on effect of self-assembly structure, Xu et al. investigated variants of WKKQLQLQLQLQLQLKK. While various self-assemblies of this peptide displayed moderate hemolytic activity, cytosis induction was found to depend strongly on their supermolecular
structure, with low hemocompatibility requiring nano-
fibers containing β-sheet structures [29]. Furthermore,
Shi et al. reported on hydrogels formed on complexes
polyoxymyxin B with different peptide amphiphiles. Lipo-
peptide release from the hydrogels was observed to span
over several days, resulting in sustained antimicrobial
effects. After P. aeruginosa infection in a burn wound
model, a 7-fold reduction in mortality was observed [30].
In a broader functional assessment, Wang et al. reported
on pH-responsive nanofiber-based hydrogels formed by
IKF8 (IKFQFHFD) for biofilm suppression and pro-
duced healing of chronic wounds. At acidic pH (char-
acteristic of infected chronic wounds), the nanofibers
were destabilized and the peptide released. In addition,
the nanofibers were loaded with cypate (a photothermal
compound) and proline (a pro-collagen compound) for
additional functionality. In vitro experiments showed
release of these compounds at pH 5.5, resulting in bio-
film elimination and subsequent activation of the healing
cascade. In vivo, complete healing of MRSA-infected
wound in mice was observed within 20 days [31].

Apart from assembly into fibers and sheets, hydro-
phobically modified AMPs may associate also to micelles
and liquid crystalline phases in a manner similar to that
of surfactants or block co-polymers. Addressing antimi-
icrobial properties of such systems, Park et al. investi-
gated self-assembly in mixtures of the antimicrobial
lipopeptide DSPE-HnMc (FKRLKKLIS-
WIKRRQQC-NH2) and PEO-PLGA. The HnMc-
containing micelles formed were found to bind to bac-
terial membranes and to display potent antimicrobial
effects for a range of bacteria. In mouse models, HnMc-
micelles were able to kill antibiotics-resistant S. aureus
and P. aeruginosa [32]. Comparing different types of
peptide self-assemblies, de Almeida et al. investigated
acylated oligosine-based amphiphiles, self-assembling
into micelles, nanofibers, and nanoribbons. It was
found that the micelles displayed potent antimicrobial
activity against MRSA and multidrug-resistant K. pneumoniae,
whereas the corresponding nanofibers were substantially less efficient. Furthermore, the anti-
microbial effects were found to depend on amino acid
composition, alkyl tail length, overall PA hydrophobicity,
and self-assembly structure, which in turn was demon-
strated to be related to the effects of these parameters
on bacterial membrane disruption [33].

**Surfactants and polar lipids**

Due to their surface activity and their ability to desta-
bilize hydrophobically driven self-assemblies (such as
phospholipid membranes) surfactants and polar lipids
may be antimicrobial, depending, e.g., on headgroup
charge and acyl chain length and structure [34]. In
addition, self-assembly structures of surfactants and
polar lipids have considerable versatility as delivery
systems for both low-Mw and biomacromolecular drugs,
including AMPs. Considering also that surfactants and
lipids provide additional functionalities of interest to
skin treatment and are widely used in medical, personal
care, and cosmetic products, it is not surprising that
such systems have received attention also as delivery
systems for AMPs in combating infection on skin and
in wounds.

Addressing AMP-loading into surfactant self-assembly
structures, Gontsarik et al. reported on self-assembly
structures formed by dioleoyltrimethylammonium-
propane (DODAP) with LL-37. Demonstrating the
responsiveness of such systems, it was found that
decreasing pH (as in infected wounds), oil-in-water
emulsions first transitioned into emulsions containing
liquid crystalline structures, and subsequently to posi-
tively charged vesicles [35]. This was explained in terms
of the protonation (and larger effective headgroup area)
of DODAP at decreasing pH. The larger headgroup area
at acidic pH, in turn, favoured encapsulation of LL-37,
as well as the resulting vesicle formation. Also address-
ning mechanisms of AMP loading into self-assembly
structures, Boge et al. investigated dispersed cubic
liquid crystalline particles (cubosomes) as carriers for
AP114 (GFGCGNPWEDDLRCHHKSIGKYGK-
GYYAAGGFVCCKC), LL-37, and DPK-060. The pep-
tides were found to differ in their degree of cubosome
association, LL-37 showing the highest degree of asso-
ciation (>60%). Mirroring this, AP114-loaded cubo-
somes displayed a largely maintained antimicrobial
effect (compared to that of the free peptide), whereas
the antimicrobial effect of LL-37 was reduced [36].

In an effort to support the development of such systems
towards therapeutics for skin and wound infections,
Boge et al. investigated cutaneous delivery of peptide-
containing cubosomes. For this, LL-37 was either
incorporated before dispersion of the liquid crystalline
phase (pre-loading), loaded into pre-formed cubosomes
(post-loading), or incorporated into cubosomes formed
spontaneously in ethanol/glycerol monooleate (hydro-
trope loading). In the case of pre-loading, no LL-37
release was observed, demonstrating strong peptide
binding to the lipid nanoparticles. In line with this,
particle-loaded LL-37 was found to be fully protected
against enzymatic degradation. No skin irritation was
observed, thus enabling for topical administration. Still,
pre-loaded LL-37 was still found to effectively kill
S. aureus bacteria in an ex vivo wound infection model
[37] (Figure 4).

In order to improve processability, long-term stability,
and other product criteria, formulations containing
AMPs may require further formulation, e.g., as dry
formulations that can display improved chemical stability
and yet be easily “activated” by hydration just prior to
delivery to the skin. Addressing this, Boge et al.
employed disaccharides to protect the liquid crystal
nanoparticles in dry powders formed by freeze-drying.
In these systems, AP114 was incorporated, and peptide encapsulation and release monitored together with antimicrobial effects \textit{in vitro}. Although the structure of the nanoparticles changed during dehydration/hydration, all freeze-dried powders were found to result in liquid crystalline particles on rehydration. Furthermore, trehalose was found to be the best cryo-protectant for maintaining a narrow particle size distribution, and for reaching a high antimicrobial activity. The release kinetics for AP114 was found to be dictated by the dimensions of the hexagonal phase, larger dimensions resulting in enhanced peptide release and resulting antimicrobial effects [38].

Addressing another type of amphiphilic self-assembly structures involving AMPs, Kumar et al. reported on a truncation of aurein 2.2 (73: RLWDIVRRWGWL). Incorporation of the peptides into PEGylated distearoylphosphatidylethanolamine micelles was found \textit{in vitro} to reduce human cell toxicity and to suppress peptide

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Glycerol monocoleate-based cubosomes were investigated as carriers for LL-37, where the peptide was incorporated either before lipid particle dispersion (pre-loading), loaded into pre-formed cubosomes (post-loading), or incorporated into spontaneously formed cubosomes in ethanol/glycerol monocoleate (hydrotrope loading). (a) LL-37 incorporation influenced the structure of the cubosomes, exemplified for pre-loaded LL-37, where SAXS results showed a transition from cubic Ia3d to lamellar structure with increased peptide loading. (b) The peptide-loaded lipid nanoparticles showed low toxicity against human keratinocytes. (c) Due to peptide incorporation into the lipid nanoparticles, susceptibility to proteolytic degradation by PE or human neutrophil elastase (HNE) was strongly suppressed. (d) A pig skin wound infection model infected with \textit{S. aureus} showed pre-loaded LL-37 to display potent antimicrobial effects (Redrawn from Ref. [37]).
Laponite induced *E. coli* aggregation at low particle concentrations (~ 5 ppm). It did not, however, lyse bacteria, but could be rendered membranolytic after LL-37 loading. (c) Aggregation of *E. coli* was triggered by laponite binding to LPS. (d) Due to the aggregation of, laponite potently suppressed NF-κB/AP-1 activation of monocytes caused by LPS in *E. coli* bacteria (Redrawn from Ref. [46]).
aggregation. Correspondingly, the free peptide aggregated when injected subcutaneously in mice, while peptides micelle-loaded peptides remained dispersed and were well absorbed. Furthermore, the micelle-loaded peptide significantly reduced abscess size and bacterial loads [39]. Investigating yet a different type of self-assembly structure, Li et al. reported on a system aimed for bacterial trapping and antimicrobial effects. In doing so, the lipopeptide C16R4 was administered in a precursor lyotropic liquid crystalline solution, forming a gel-like structure after administration. The latter, in turn, was able to trap bacteria at the site of infection. Employing an animal model in which MRSA was inoculated subcutaneously in mice, the antibacterial performance was found to be significant [40].

**Inorganic nanoparticles**

While formulations formed by: (i) polymer chains, self-assemblies or fibers, (ii) self-assemblies formed by surfactants or polar lipids, and (iii) peptide self-assemblies, as well as dry polymer-based fiber mats, are the most widely investigated types of AMP delivery systems so far, increasing attention has recently been placed on additional features which can be provided by various inorganic nanoparticles. Such additional functionalities include, e.g., photocatalytic or photothermal effects, or efficiency in flocculating bacteria and inflammatory bacterial components.

**Mesoporous silica nanoparticles**

Due to their well-defined pores, mesoporous silica nanoparticles are interesting as AMP carriers. Addressing the mechanistic foundation of this, Braun et al. investigated effects of porosity and charge on AMP loading and release, as well as on membrane interactions and antimicrobial effects of such systems [41]. Demonstrating the role of electrostatics, anionic particles incorporated much more of LL-37 than the corresponding positively charged ones. Due to pore peptide loading into the pores of the nanoparticles, anionic particles were able to protect LL-37 from proteolytic degradation. For anionic nanoparticles, membrane disruption occurs primarily after peptide release. In contrast, non-porous particles bound LL-37 strongly due to their higher negative charge, causing membrane interactions and antimicrobial effects to be largely mediated by the peptide-coated nanoparticles. For positively charged particles, LL-37 incorporation promoted bacterial membrane disruption, but also caused adverse effects in terms of hemolysis.

Further demonstrating the importance of nanoparticle properties on their performance as AMP delivery system, Malekhiat-Häffner et al. compared smooth and virus-like mesoporous nanoparticles, the latter having a “spiky” external surface [42]. While the virus-like particles were able to destabilize bacteria-like lipid membranes, the corresponding smooth nanoparticles were not, demonstrating the importance of particle topology for membrane destabilization, an effect accentuated further after loading particle loading with LL-37. Based on neutron reflectometry, it was demonstrated that the virus-like nanoparticles induce trans-membrane defects and promote LL-37 binding throughout the membrane. The relevance of such effects of particle spikes for bacterial membrane rupture was also demonstrated for *E. coli* bacteria.

Although silica is the type of mesoporous nanoparticles most widely investigated as AMP delivery systems, also others are receiving increasing attention. Exemplifying this for skin infection, Flynn et al. investigated nisin A loaded into mesoporous silicates and periodic mesoporous organosilanes [43]. Hydrophobic interactions were found to be key for peptide loading into both these types of matrices. Furthermore, both types of mesoporous materials were able to protect nisin A from pepsin degradation. Finally, no peptide release was observed at pH 6.5, at the same time as the peptide-loaded matrices were active against *S. aureus*, indicating such effects to be mediated by the peptide-loaded nanoparticles. Taken together, there thus seem to be similarities between mesoporous silica and organosilanes as AMP delivery systems.

Reporting on a more advanced AMP delivery system, Yu et al. investigated host mesoporous silica nanoparticles capped by β-cyclodextrin and corresponding guest adamantane-decorated nanoparticles containing a magnetic core capped [44]. Together, these formed co-assemblies based on cyclodextrin-adamantane interactions. The supramolecular co-assemblies thus formed released loaded melittin (GIGAVLKVLTTGGLPALISWKRLDVQ) and ofloxacin on heating, resulting in higher suppression of *P. aeruginosa* PA01 biofilms compared to the free drugs and empty nanoparticles, with limited cell toxicity. On blocking the cyclodextrin-adamantane interaction, the antibiofilm activity was strongly suppressed. Also *in vivo*, the co-assemblies were efficient in eradicating bacterial biofilms preventing tissue damage and inflammation.

**Nanoclays**

Nanoclays are plate-like nanomaterials, very efficient in interacting with bacterial membranes, and as flocculants of bacteria and bacterial components. Addressing the mechanistic foundation for this, Malekhiat-Häffner et al. investigated net cationic layered double hydroxides (LDHs) of different size. As a result of an increasing edge fraction, LDH binding to bacteria-like lipid membranes, anionic lipid extraction, and membrane lysis were all promoted by decreasing particle size, an effect remaining after LL-37 loading. Analogously,
Sułek et al. reported porphyrin-doped TiO₂ nanoparticles’ antimicrobial effect on illumination. For example, the controllable band gap of TiO₂ can be strongly decreased by loading laponite with LL-37 (Figure 5). Due to aggregation of both bacteria and free lipopolysaccharide (LPS), laponite suppressed NF-κB activation of monocytes caused by bacteria and LPS [46]. Thus, AMP-loaded nanoclays represents an approach for infection confinement, similar to the peptide-based one playing a central role in wound healing [47].

**Photocatalytic nanomaterials**

In photocatalysis, light excitation triggers the formation of free electrons and positively charged holes, which may react to form reactive oxygen species (ROS) at the nanoparticle surface. ROS, in turn, may oxidize membrane and other essential components in bacteria [48]. TiO₂ was among the first photocatalytic nanomaterials to be investigated. Recently, carbon-based photocatalysts have received much attention due to their facile synthesis and functionalization, and wide and controllable band gap. Photocatalysts can induce dramatic antimicrobial effect on illumination. For example, Sulek et al. reported porphyrin-doped TiO₂ nanoparticles to result in a 7-log reduction of *S. aureus* under illumination [49], while Ahmed et al. reported potent antimicrobial effects of TiO₂ nanoparticles on 25 MDR *P. aeruginosa* strains [50].

In an attempt to elucidate the mechanism of photocatalytic degradation of bacterial membranes by TiO₂ nanoparticles, Malekkhhaia-Haffner et al. reported on effects lipid membrane composition, demonstrating that anionic POPG promoted photocatalytic degradation, while cholesterol provided a stabilizing effect [51]. Considering the partial preference for degradation of bacteria-like anionic membranes over zwitterionic and cholesterol-containing ones already of bare TiO₂ nanoparticles, as well as the possibility to further boost targeting to bacteria membranes through AMP loading, photocatalytic TiO₂ nanoparticles may have potential as carrier of AMPs for boosted antimicrobial effects. Somewhat related, Chen et al. studied antimicrobial properties of similarly photocatalytic ZnO nanoparticles coated by the AMP UBI29-41, and found the latter to facilitate membrane destabilization, thus enhancing the antimicrobial effect of the co-loaded antibiotic vancomycin against *B. subtilis* and *S. aureus* [52].

Fullerenes are cage-like carbon structures, able to destruct bacterial membranes both by direct membrane binding and ROS-mediated degradation. Addressing the therapeutic consequences of such effects, Lu et al. found cationic C₆₀ to induce potent antimicrobial effects under illumination [53]. In wounds infected with either *P. aeruginosa* or *Proteus mirabilis*, illumination in the presence of C₆₀ strongly suppressed bacteria, which correlated to a dramatic improvement in survival (from 8% to 82%) compared to no treatment. While fullerenes as AMP delivery systems remain to be investigated, Dostalova et al. reported maximin H5 (ILGPVLGLVDTDDVLGIL-NH₂) variants loading to C₆₀ found the composite particles to display strongly boosted antiviral activity compared to free peptide [54]. Analogously, Ren et al. reported D28 (FLGVVFK LASKVFPAVFGKV) conjugated to graphene oxide (GO) to displays much stronger antimicrobial activity than bare GO [55].

**Photothermal nanomaterials**

Some nanomaterials are able to generate localized heat, e.g., on excitation by light or oscillating magnetic fields. Due to localized heat generated in such processes, potent antimicrobial effects are provided [57,58]. In addition to having antimicrobial effects on their own, such systems are also interesting as AMP delivery systems, where that may provide a range of functional advantages. Illustrating this, Comune et al. reported on wound healing by LL-37, either free or immobilized on AuNP. AuNP-loaded LL-37, but not free peptide, was found to enhance keratinocyte migration. Mirroring this, nanocarrier-loaded LL-37 displayed improved wound healing *in vivo* compared to free peptide [59]. Furthermore, Chen et al. investigated on gold nanodots loaded with surfacitin. The nanodots obtained were very small (≈ 2.5 nm) and highly efficient antimicrobials. For example, the minimal inhibitory concentration of the surfacitin-loaded nanodots was >80-fold lower than that of the free peptide. At the same time, *in vitro* cytotoxicity and hemolysis analyses showed the nanodot/peptide system to be better tolerated than the free peptide. *In vivo*, MRSA-infected wounds in rats showed faster healing for the peptide-loaded nanodots [60].
Antimicrobial effects of a NIR-triggered antibacterial system, containing a MIL-101 core, loaded with vancomycin and coated with LL-37. In the presence of the ROS precursor H$_2$O$_2$, Fenton-like reactions resulted in the formation of hydroxyl radicals (\(\cdot\)OH) (a), which in turn oxidized essential bacterial components and resulted in bacterial membrane rupture, as indicated by the release of intracellular proteins (b). (c) Despite being potently antimicrobial, the peptide-loaded MIL-101 displayed low cell toxicity. (d) At endogenous H$_2$O$_2$ overexpression and acid conditions (as in bacterial infections), in vivo suppression of MRSA in wounds of KM mice was demonstrated, as was improved wound healing (Redrawn from Ref. [56]).
Employing photothermal effects, Dong et al. investigated a hybrid system, formed by Au/Ag nanorods, and the lipopeptide daptomycin, as well as its antimicrobial effects on MRSA [61]. In the presence of H₂O₂, the hybrid material released silver ions and daptomycin, disintegrating the bacteria membrane. On laser irradiation, results both in vitro and in vivo showed improved antibacterial activity for gold nanorods with good photothermal effect. However, provided that laser irradiation was applied at the initial stage of infection and the temperature controlled (<47 °C), MRSA growth could be inhibited, thereby preventing ulceration and promoting wound healing, with no thermal damage to the wound or the surrounding skin (Figure 7). Similarly, Chen et al. investigated a hybrid system formed between BF2b (CCRAGLQFPVGRLLRRLLR) and gold nanorods, as well as its antimicrobial effects against MRSA [62]. The peptide-loaded nanorods had a positive surface change, providing high affinity for bacterial membranes. On NIR irradiation, potent antibacterial performance against MRSA was observed both in vitro and in vivo. Analogously, Fu et al. reported on poly(lysine)-coated phosphorous nanosheets, and found these cationic nanoparticles to bind to, and lyse, negatively charged bacteria. Adding to this, NIR irradiation caused photothermal heating, further boosted antimicrobial potency. In vitro, antibacterial effects against MRSA were observed within 15 min. Analogously, in vivo experiments in showed a 99.4% suppression of MRSA in a mice skin infection model, at the same time as toxicity was low [63].

Outlook

From the discussion above, it seems clear that delivery systems for AMPs have the potential to successfully combat challenging bacterial infections in wounds and impaired skin, and to reduce problems with infected, non-healing, and chronic wounds, including those caused by bacteria resistant to conventional antibiotics. This is not to say, however, that resistance cannot develop for AMP-based systems as well. Indeed, several different mechanisms for resistance development of bacteria against AMPs have already been identified, including, e.g., upregulation of glucose amino glycans, alginate, and other polyanionic compounds able to scavenge AMPs, upregulation of proteases able to degrade AMPs, and lipid headgroup modification (reducing affinity for such membranes), to mention a few [64]. Many of these, however, require major adaptations of the bacteria, and often come at the price of reduced fitness [65]. Nevertheless, development of AMPs into therapeutics for combatting infected skin and wounds requires additional focus to be placed on resistance development for such systems. Protocols for studies of resistance development have been widely applied for conventional antibiotics, but much less so for AMPs, and hardly at all for AMP-delivery system combinations. It is therefore of key importance for the development of AMPs towards therapeutics that such effects are increasingly investigated. Closely related to this, the spectral width of antimicrobial effects remains a sparsely investigated field of AMPs in general, and of AMP-delivery systems in particular. More work is therefore needed to evaluate AMPs, and particularly so together with delivery systems, for a wider range of bacteria, and with a larger focus on challenging clinical isolates and MDR strains.

A definitive advantage with skin and wound infections regarding AMP delivery is the relative accessibility of the infected tissue to delivery systems and AMPs alike. This, in turn, enables local administration of therapeutics (as opposed to systemic administration, e.g., via parenteral, oral, or pulmonary routes). This, in turn, eliminates problems with systemic adverse effects and reduces the amount of AMPs needed to combat infections. This is favourable from a cost-of-goods perspective, making topical administration one of the most likely areas for AMPs to be sufficiently affordable to allow their development into therapeutics. From a resistance perspective, the lower doses typically required for treating skin infections by topical delivery will also lower the development pressure for resistance. Furthermore, since skin and wound infections are superficial, potent antimicrobial effects provided by photocatalytic or photothermal nanomaterials may be combined with AMPs as delivery systems and co-antimicrobials, further opening opportunities for combatting challenging infections. For such hybrid systems to be developed, however, additional work is needed on the interplay between such nanomaterials and AMPs, e.g., related to how AMP loading affect photocatalytic and photothermal effects and vice versa, and how such AMP-loaded functional nanoparticles interact with bacterial membranes and lipopolysaccharides in the absence and presence of illumination. Furthermore, since some AMP-loaded drug delivery systems represent intermediates between a Class 3 medical device and a drug product, they need to be regulated regarding aspects related to AMP nanoparticle, the hybrids generated, as well as to the type and degree of illumination for photocatalytic/photothermal nanomaterials. Considering this, one could expect such systems to appear first in more regulated settings, e.g., in hospital-localized treatments of burn wounds or the like.

Finally, while focus of the present overview has been placed on wounds, there are a wide range of indications where infected skin remains intact (although impaired), including, e.g., atopic dermatitis (AD) [66]. Uncontrolled bacterial infection constitutes a key feature of these indications as well, and AMPs represent an interesting approach for addressing these, particularly as these patients typically suffer from lower than natural endogenous AMP levels. For such patients, AMP
Antimicrobial effects of Au/Ag nanorods, loaded with the lipopeptide daptomycin, against MRSA. (a) In the presence of the ROS precursor \( \text{H}_2\text{O}_2 \), the hybrid material released silver ions and daptomycin, resulting in antimicrobial effects, which was further promoted on laser irradiation. (b) Release of intracellular proteins and DNA demonstrate these effects to be correlated with bacterial membrane disruption. (c) If laser irradiation was given at the initial stage of infection and the temperature controlled (<47 °C), MRSA growth was suppressed and wound healing promoted (Redrawn from Ref. [61]).
therapies would therefore essentially aim to restore the AMP function, which is present in healthy individuals but compromised in AD patients. Although the skin barrier function of AD patients is impaired, it still provides a formidable challenge for the delivery of large and hydrophilic molecules such as AMPs [67]. As a consequence, medical device approaches such as microneedle patches, iontophoresis, sonophoresis, and the like all have a place in this context. However, the broader use of AMPs as therapeutics to treat AD and related indications (often spread out over large areas of the skin) requires the development of delivery systems able to overcome the stratum corneum. Here, inspiration can be drawn from delivery systems which have previously been efficient in delivering low-Mₘ drugs over the stratum corneum, such as microemulsions, various types of structured lipid nanoparticles and other self-assembly systems [68]. Such delivery systems therefore need further attention in relation to AMP delivery over the skin barrier in combatting infection and inflammation in AD and other diseases with impaired but still partially intact skin barrier function.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability
No data was used for the research described in the article.

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References
Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


Nice demonstration of how AMPs can be included in DNA hydrogels for reduced toxicity at maintained antimicrobial effects, also ex vivo for antibiotics-resistant bacteria.


A nice demonstration of wider effect spectrum of AMP-loaded fibers, including not only antimicrobial effects, but also immunomodulatory ones.

22. Teixeira MA, Antunes JC, Seabra CL, Tohidi SD, Reis S, Amorim MTP, Felgueiras HP: Tiger 17 and pexiganan as anti-microbial and hemostatic boosters for cellulose acetate-


NICE demonstration of the ability of AMP-loaded electrop spun fibers to display potent antimicrobial effects, also against drug-resistant and biofilm-forming bacteria, ranging from comparing two approaches for AMP loading, via in vitro studis on antimicrobial effects and cell toxicity, to performance evaluation in vivo.


A nice demonstration of pH-dependent AMP self-assembly and disassembly, which may be loaded with photothermal and antimicrobial agents, and together with these deliver potent antimicrobial effects.


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A systematic investigation into the effect of structures formed on membrane interactions and antimicrobial effects of acylated peptide amphiphiles.


Nice study coupling structural aspects of AMP loading to, and release from, cubosomes with functional performance of such systems regarding antimicrobial effects and cell toxicity in skin infection models.


A nice investigation into a lipopeptide-based lyotropic liquid crystalline phase that can form a “spounge” to effectively capture and kill bacteria, demonstrated to work well wounds infected by drug-resistant bacteria in vivo.


A systematic investigation into mechanisms of AMP-loading to, and release from, mesoporous silica nanoparticles, demonstrating that surface spikes on the surface of such nanoparticles work in concert with antimicrobial peptides for boosted membrane destabilization and antimicrobial effects.


Demonstration of LPS-mediated binding of nanoclays to bacteria and resulting co-aggregation, which results in suppression of LPS-induced NF-xB activation in monocytes, thus devising an approach for confining infection and inflammation in wounds.


A systematic investigation into mechanisms underlying photocatalytic degradation of lipid membranes of different composition, providing information on effects of anionic phospholipids and cholesterol, key components in membranes of bacteria and human cells, respectively.


Nice demonstration of the interplay between direct membrane action and photocatalytic effects, as well as its consequences for antimicrobial effects and improvement of wound healing for antibiotics-resistant bacteria.


Nice demonstration of synergistic antimicrobial effects displayed by AMP-loaded photothermal gold nanoparticles, as well as its consequences for improving wound healing.


Nice demonstration of the interplay between direct membrane action and photothermal effects, as well as its consequences for antimicrobial effects and improvement of wound healing for antibiotics-resistant bacteria.


