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Antimicrobial peptides – Unleashing their therapeutic potential using nanotechnology

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ABSTRACT

Antimicrobial peptides (AMPs) are potent, mostly cationic, and amphiphilic broad-spectrum host defense antimicrobials that are produced by all organisms ranging from prokaryotes to humans. In addition to their antimicrobial actions, they modulate inflammatory and immune responses and promote wound healing. Although they have clear benefits over traditional antibiotic drugs, their wide therapeutic utilization is compromised by concerns of toxicity, stability, and production costs. Recent advances in nanotechnology have attracted increasing interest to unleash the AMPs' immense potential as broad-spectrum antibiotics and anti-biofilm agents, against which the bacteria have less chances to develop resistance. Topical application of AMPs promotes migration of keratinocytes and fibroblasts, and contributes significantly to an accelerated wound healing process. Delivery of AMPs by employing nanotechnological approaches avoids the major disadvantages of AMPs, such as instability and toxicity, and provides a controlled delivery profile together with prolonged activity. In this review, we provide an overview of the key properties of AMPs and discuss the latest developments in topical AMP therapy using nanocarriers. We use chronic hard-to-heal wounds—complicated by infections, inflammation, and stagnated healing—as an example of an unmet medical need for which the AMPs' wide range of therapeutic actions could provide the most potential benefit. The use of innovative materials and sophisticated nanotechnological approaches offering various possibilities are discussed in more depth.

Contents

Abbreviations: A3-APO, proline rich peptide; AMR, antimicrobial resistance; AMP, antimicrobial peptide; AuNP, gold nanoparticle; CH, chitosan; CHNPs, chitosan nanoparticles; ECM, extracellular matrix; EDC, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FPRL1, formyl peptide receptor-like 1; GMO, Glycerol monooleate; HaCaT, immortalized human keratinocyte cell line; hCAP18, human cationic antimicrobial protein; hBD, human β defensin; HDF, human dermal fibroblasts; HNP, human neutrophil peptide; IGF1, insulin-like growth factor 1; IL-1β, -6 and -8 interleukins 1β-6 and -8; KR12, antimicrobial motif (residues 18–29) of the human cathelicidin peptide; LL-37, 37-amino-acid-long C-terminal human cathelicidin peptide; LCNP, liquid crystalline nanoparticle; LPS, lipopolysaccharide; MA, methacrylamide; MAPK, mitogen-activated protein kinase; MDR, multidrug resistance; MPO, myeloperoxidase; MRSA, methicillin-resistant *S. aureus*; NFκβ, nuclear factor-kappa B; NHS, N-hydroxysuccinimide; NP, nanoparticle; OH30 peptide, cationic peptide from the king cobra; PEG, polyethylene glycol; PCL, polycaprolactone; PI3K, phosphoinositide-3-kinase; PLA, polylactic acid; PLGA, polylactic-co-glycolic acid; PLGA NP, poly (lactic-co-glycolic acid) nanoparticle; PR-39, porcine proline-rich antimicrobial peptide; PVA, polyvinyl alcohol; ROS, reactive oxygen species; S100A7, psoriasin; SEM, scanning electron microscopy; SF, silk fibroin; SLN, solid lipid nanoparticle; TEM, transmission electron microscopy; TGFα, transforming growth factor-alpha; TGFβ, transforming growth factor-beta; TLR, Toll-like receptors; TNFα, tumor necrosis factor α;; VEGF, vascular endothelial growth factors.

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1. Introduction

Antibiotics, from their discovery in the 1940s onwards, have revolutionized medicine and provided cures for several serious or fatal infectious diseases. Unfortunately, their widespread, uncontrolled, and irrational use has promoted the development of drug-resistant microbial strains. The increasing presence of resistant pathogens contributes to a prolonged and complicated disease course, and eventually treatment failures, thus compromising our seemingly successful fight against infections (Ventola, 2015). Furthermore, the growth of pathogenic microorganisms as biofilms, complex communities of microbial cells enclosed in their self-produced matrices, increases the pathogens' ability to repel and tolerate antimicrobial regimens. This fuels infections to become chronic, untreatable, and fatal. The public health threat of increasing antimicrobial resistance (AMR) urgently warrants the development of new antibiotics and therapies to fight microbial infections, disrupt biofilms, and prevent any opportunistic infections arising from antibiotic therapy-induced breakdown in the balance of a healthy and diverse microbial ecosystem (Bjarnsholt, 2013).

Skin wounds are practically always contaminated with microbes, but infection occurs only when the host defense mechanisms are overwhelmed by microbial virulence and growth (Bowler, Duerden, & Armstrong, 2001). Wound colonization is usually polymicrobial and combines aerobic and anaerobic microorganisms. The leading bacterial species present in wound infections are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and enterobacteria* (Berríos-Torres et al., 2017). Prolonged inflammation, recurrent microbial infections, and drug-resistant biofilms complicate chronic non-healing wounds (Rahim et al., 2017). From the beginning of the millennium, wound infections have been increasingly populated by methicillin-resistant *S. aureus* (MRSA). This has caused worldwide surges in wound complications such as progressive necrotizing damage to the surrounding tissue and bacteremia (Demling & Waterhouse, 2007).

Antimicrobial peptides (AMPs), also termed host defense peptides (HDPs), are widely expressed in prokaryotes and eukaryotes (Huan, Kong, Mou, & Yi, 2020). Some of the common motifs of AMPs have been dated back in evolution at least 500 million years (Zhu & Gao, 2013). In epithelia, AMPs are produced in response to pathogens to preserve surface integrity and prevent any opportunistic infections in the host. In skin cells, and especially in epidermal keratinocytes, the production of AMPs is induced in response to microbial stimuli (Brandwein, Bentwich, & Steinberg, 2017). Thus, in infected wounds, AMPs, such as defensins and cathelicidins, are a crucial part of the skin's active defense mechanism (Pasupuleti, Schmidtchen, & Malmsten, 2012). Moreover, AMPs have also immunomodulatory actions and aid in wound healing (Pfalzgraff, Brandenburg, & Weindl, 2018)—a multi-level cascade involving hemostasis, inflammation, and tissue remodeling (Rousselle, Montmasson, & Garnier, 2019). Beginning from the very first identification of an AMP, lysozyme by Alexander Fleming in the 1920s, the therapeutic potential and applications of AMPs have fascinated both the medical and scientific communities. However, the therapeutic efficiency of AMPs is considerably compromised by their rapid degradation and cytotoxicity at high concentrations.

In this review, we guide the reader through the actions of AMPs by using wounds, especially chronic hard-to-heal wounds, as an example. Such stagnated wounds are usually complicated by perturbations in infection clearance and inflammation control, and as such they are well suited for deconstructing the variety of actions of AMPs. Moreover, we review the promising current advances in nanotechnology for the therapeutic utilization of AMPs.

2. AMPs in the human skin host defense response: defensins and cathelicidins

Skin acts as a physical barrier against pathogens (Brandwein et al., 2017). AMPs are widely expressed in human skin under normal conditions. As key effectors of the innate immune system, their expression is induced in response to tissue damage or infections (Fig. 1). Two major categories of AMPs are found in humans: defensins and cathelicidins (Ganz, 2003). The defensins belong to the protective class of AMPs found in keratinocytes, but also in epithelial cells of the gut and respiratory-tract. The eukaryotic defensins can be divided according to their structure into four groups containing 1) cysteine-free helices, 2) extended cysteine-free helices, 3) loop structures with one disulfide bond, or 4) sheet structures with two or three disulfide bonds. The defensins found in mammals belong to the latter group. They are 18–45 amino acid long, and are further classified into α, β, or θ subcategories according to their molecular structural differences. In general, their three disulfide bonds between six cysteine residues provide the basis for distinctive surface and molecular interactions as well as antimicrobial activity against different microorganisms (Schneider, Unholzer, Schaller, Schäfer-Korting, & Korting, 2005). The α defensins, also known as neutrophil peptides, were originally characterized in bovine polymorphonuclear leucocytes (Selsted et al., 1993). In humans, there are five α defensin genes that produce six types of human neutrophil peptides, HNP 1–6. The first four peptides (HNP1–4) are present in neutrophils while HNP5 and 6 are located in the Paneth cells of the small intestine (Lehrer & Lu, 2012). The human β-defensins (hBDs), on the other hand, are four in number and, interestingly, are activated in different layers of the skin. The human β-defensin 1 (hBD1) was first found in a plasma filtrate from renal disease patients (Bensch, Raida, Mägert, Schulz-Knappe, & Forssmann, 1995). It is expressed constitutively and almost ubiquitously in all epithelia, including epithelia of the skin, salivary glands, oral mucosa, urogenital tract, trachea, cornea and colon. The hBD2 and 3 were recovered from the flakes of psoriatic skin representing their role in defense and, besides skin, are also expressed in the gastrointestinal and respiratory tracts (Harder, Bartels, Christophers, & Schröder, 1997). High expression of hBD4 was identified in neutrophil granulocytes, urogenital tissues, stomach and thyroid. The hBD2 is inducible by inflammatory (e.g. interleukin-1) and bacterial stimuli (e.g., live *Pseudomonas aeruginosa*). Moreover, the state of cell differentiation (Liu et al., 2002) and inflammatory activation of lymphocytes or monocytes can affect hBD2 production (Sørensen et al., 2005). hBD3, on the other hand, is inducible selectively by interferon-γ. All hBDs promote chemotaxis of leukocytes, but differ in their cell type selectivity (Röhrl, Yang, Oppenheim, & Hehlgans, 2010) (García et al., 2001). Production of hBD2–4 accelerates wound healing through epidermal growth factor receptor (EGFR)-stimulated keratinocyte migration (Niyonsaba et al., 2007).

Cathelicidins are a group of small cationic AMPs that vary by their amino acid structure, size, and sequence. Although approximately thirty cathelicidins have been identified in mammals, only one cathelicidin has been discovered in humans (Kościuczuk et al., 2012). The cathelicidins are generally produced by neutrophil granulocytes (Sørensen, Arnljots, Cowland, Bainton, & Borregaard, 1997). For activation, they require cleavage and release of the functional C-terminal fragments (Vandamme, Landuyt, Luyten, & Schoofs, 2012). The human

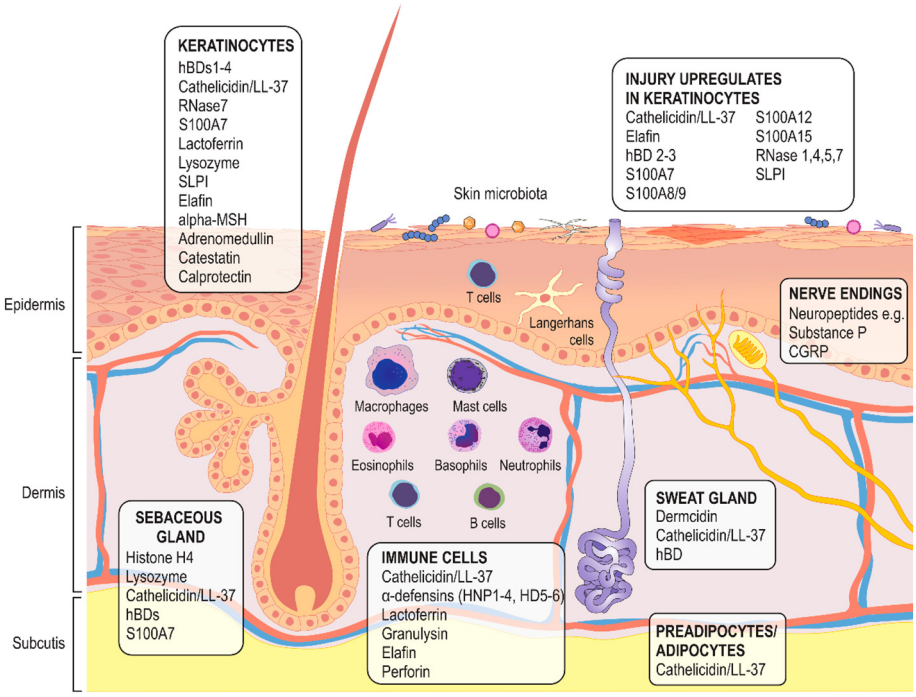

Fig. 1. Distribution of different antimicrobial peptides (AMPs) across human skin (Nakatsuji & Gallo, 2012). Key: hBD, human β defensin; HNP, human neutrophil peptide; CGRP, calcitonin gene-related peptide; SLPI, secretory leucocyte protease inhibitor; alpha-MSH, α-melanocyte-stimulating hormone; S100A7, psoriasin; S100A8/9, calprotectin; S100A15, koebnerisin; S100, S100 family peptides with calcium-binding sites.

cathelicidin, cationic antimicrobial protein (hCAP18), is cleaved by serine proteases (Kahlenberg & Kaplan, 2013) to release its 37-amino-acid-long C-terminal peptide called LL-37 (Kościuczuk et al., 2012; Sørensen et al., 2001). LL-37 forms an amphipathic helical structure and acts as broad-spectrum antimicrobial (Turner, Cho, Dinh, Waring, & Lehrer, 1998). The peptide is mainly located in the epithelia of the skin, gastrointestinal and respiratory tracts as well as in inflammatory cells. LL-37 helps in controlling infection through antimicrobial action by binding to and neutralizing lipopolysaccharides (LPS) and modulating the inflammatory process (Dürr, Sudheendra, & Ramamoorthy, 2006). Mice with deletions in cathelicidin genes (e.g. *Camp* and *Cnlp*) show poor skin immune responses and defense against skin infections e.g. by the group A *Streptococcus* (GAS) (Nizet et al., 2001).

The cathelicidin LL-37 and hBDs are important functional components of our innate immune system and are active against a wide array of skin pathogenic microorganisms (Reinholz, Ruzicka, & Schauber, 2012). The absence or deficiency of these effector molecules contributes to the development of such skin conditions as atopic dermatitis, eczema and psoriasis (Ong et al., 2002).

Apart from defensins and cathelicidin, many other antimicrobial molecules also provide immunity in skin infections, including proteinase inhibitors, chemokines, neural proteins/peptides, neuropeptides, and enzymes. For example, RNAase 7 is a 14.5 kDa antimicrobial ribonuclease active towards Gram-positive and Gram-negative bacteria as well as fungi (Harder & Schröder, 2002). Dermcidin, on the other hand, is a 47 amino acid peptide secreted by sweat glands and transported to epidermal surface. It has also been shown to have antimicrobial activity against both microbial and fungal skin infections (Schittek et al., 2001). Inflammatory conditions, such as psoriasis, can lead to the production and expression of psoriasin (S100A7) in keratinocytes (Algermissen, Sitzmann, LeMotte, & Czarnetzki, 1996). The expression of S100A7 and other S100 protein family members is found in the epidermis as well as scattered across the sebaceous glands and hair follicles (Eckert et al., 2004; Alowami, Qing, Emberley, Snell, & Watson, 2003). S100A7 exerts its bactericidal activity by sequestration of Zn^{2+} (Lee & Eckert, 2007) or pH-dependent permeabilization of bacterial membranes (Michalek et al., 2009). It also increases production of chemotactic agents that stimulate migration of neutrophils and T-lymphocytes (Jinquan et al., 1996). Sequestration of zinc-ions has also been described as the basis for antimicrobial activity of S100A8/A9, calprotectin (Sohnle, Hunter, Hahn, & Chazin, 2000). In concert with these findings, other members of the S100 protein family have been shown to harbor antimicrobial and immunostimulatory activities against invading microorganisms as well (Zackular, Chazin, & Skaar, 2015). The enzyme, lysozyme (14–15 kDa) is a peptidoglycan hydrolase present in human skin. It is abundant in the epidermis and provides the human skin with gram positive bacterial cell wall peptidoglycan breakdown activity of approximately 85 and 195 μg/g wet weight (Niyonsaba & Ogawa, 2005; Papini, Simonetti, Franceschini, Scaringi, & Binazzi, 1981). Interestingly, lysozyme-derived peptides have also been shown to have antibacterial properties independent of lysozyme enzymatic activity (Nash, Ballard, Weaver, & Akinbi, 2006). The iron-binding glycoprotein lactoferrin, secreted by glandular epithelial cells in the skin, exhibits anti-inflammatory and anti-infective properties (Takayama & Aoki, 2011), and its expression has been reported to be induced in keratinocytes and lymphocytes during an acute phase innate immune reaction (Lindford et al., 2021). Inflammatory reactions are also regulated by neuronal activity, for example through the production of neuropeptides that cause vasodilation, plasma extravasation, and inflammatory cell recruitment (Luger & Lotti, 1998). The neuropeptides substance P, calcitonin gene-related peptide, neuropeptide Y, and vasoactive intestinal peptide have been demonstrated to have antimicrobial activity (El Karim, Linden, Orr, & Lundy, 2008).

Released at sites of extensive cellular damage (Silk, Zhao, Weng, & Ma, 2017), extracellular histones work together with AMPs, such as LL-37, to affect bacterial membrane permeability, DNA or RNA binding, LPS binding, and killing of microorganisms by neutrophil extracellular traps (NETs) (Doolin et al., 2020; Kawasaki & Iwamuro, 2008). As our innate immune system's first responder cells, the neutrophil granulocytes have evolved multiple ways of microbial killing. They target their

actions both extracellularly and to specific granules, that upon fusion, concentrate antimicrobial actions on intracellular phagosomes. Neutrophils also produce reactive oxygen species (ROS), AMPs, and with the help of myeloperoxidase (MPO) enzymes produce toxic antimicrobial intermediates to fight infections (Nauseef, 2014). NETosis is a specific form of cell death or self-sacrifice of neutrophils (Thiam, Wong, Wagner, & Waterman, 2020). It results in the formation of cobweb-like NETs enriched with antimicrobial activity, including histones, AMPs, and MPO (Brinkmann et al., 2004). Biological utilization of AMPs suggests that they are most active when targeted at high concentrations and acting in synergism with other antimicrobial approaches.

3. Immunomodulatory actions of AMPs

In addition to their activity towards outside invaders, AMPs also modify many host responses. They have dominant effects on the immune system and in repair of tissue damage. Such activities of AMPs involve the modulation of inflammation signaling pathways, T-cell responses, differentiation of macrophages and dendritic cells, wound repair (Heilborn et al., 2003) and apoptosis (Barlow et al., 2010). Specifically, one identified mechanism of AMPs involves suppression of LPS-induced inflammation and reduction of NFκβ activity as well as cytokine secretion (Håversen, Ohlsson, Hahn-Zoric, Hanson, & Mattsby-Baltzer, 2002). Moreover, AMPs can form complexes with immune ligands such as double-stranded nucleic acids, single-stranded RNA or bacterial surface molecules to modulate or enhance their receptor-mediated host cell responses. Several down-stream immunomodulatory activities have been assigned to AMP binding to either immune cell receptors or immune ligands (Lee, Lee, & Wong, 2019). These include regulation of phagocytic activity (Wan et al., 2014), effector-molecule recruitment, chemotaxis (Tjabringa, Ninaber, Drijfhout, Rabe, & Hiemstra, 2006), bacterial killing (Lee et al., 2019) and endotoxin-neutralizing activity. Importantly, AMPs can counterbalance excessive and damaging, even systemic, proinflammatory responses (Mahlapuu, Håkansson, Ringstad, & Björn, 2016).

Complex inter-regulatory networks contribute to a successful host defense, controlled inflammatory activity and immune response. AMPs participate in this regulation by interacting with the receptors of immune-effectors, growth factors and chemokines (Fig. 2). Reciprocally, certain growth factors, such as insulin-like growth factor 1 (IGF1) and transforming growth factor-alpha (TGFα), stimulate the expression of AMPs, for example LL-37 and hBD-3 (Pivarcsi, Nagy, & Kemeny, 2005; Seeger & Paller, 2015). LL-37 has profound effects in controlling the LPS induced pro-inflammatory mediators and combating infection through 1) inhibition of LPS-facilitated nuclear factor kappa beta (NFkβ); 2) altering the transcription of proinflammatory cytokines and other proinflammatory genes; 3) modulation of the activation of mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) signaling pathways; 4) inactivation of LPS through direct binding and neutralization; and 5) decreasing the LPS-induced production or secretion of pro-inflammatory mediators TNFα and IL6 (Hilchie, Wuerth, & Hancock, 2013).

The evolutionarily conserved nature of AMPs manifests as their activity across species. AMPs from other animals, insects, and microorganisms as well as AMPs generated through chemical synthesis have immunomodulatory effects in humans. For example bovine lactoferrin and microbial nisin A have been shown to suppress inflammatory reactions in LPS-activated human keratinocytes, endothelial cells and monocytes (Mattsby-Baltzer et al., 1996; Mouritzen, Andrea, Qvist, Poulsen, & Jenssen, 2019). In general, AMPs exert dominant control over immunological responses at the interface of innate and adaptive immunity in an interspecies conserved manner (Lai & Gallo, 2009), and thus a wide variety of AMPs hold therapeutic potential for the treatment of chronic wounds and wound infections.

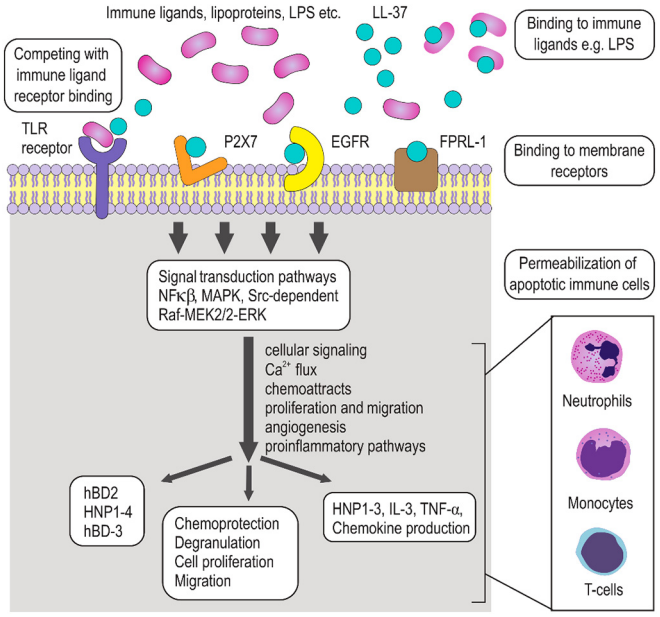

Fig. 2. Representation of immunomodulation and cellular cross-talk with mediators and receptors by antimicrobial peptides (AMPs) during wound healing. The inflammation occurs by formyl peptide receptor-like 1 (FPRL1) receptor acting as chemoattract for neutrophils, monocytes, and T cells (Yang et al., 2000; Tjabringa et al., 2003). For example, the LL-37 AMP acts in various ways in the host immune system. These include direct binding to immune ligands, competition of immune ligand receptor binding and binding to surface receptors, such as FPR2 (FPRL-1), EGFR, and ERBb2 (Kuroda, Okumura, Isogai, & Isogai, 2015). The permeability of apoptotic leukocytes controlled by LL-37 can result in shifts of proinflammatory signals during the inflammatory process (Björstad et al., 2009). Key: TLR, Toll-like receptors; LPS, lipopolysaccharide; P2X7, P2X purinoceptor 7; EGFR, *epidermal growth factor receptor;* FPRL-1, formyl peptide receptor-like 1; MAPK, mitogen-activated protein kinase; hBD-2/3, human β defensins; HNP1-4, human neutrophil peptides; NFκβ, nuclear factor-kappa; Ca^{2+}, calcium; TNF α, tumor necrosis factor-alpha; Src-dependent Raf MEK1/2 ERK pathway, signaling pathway following RAF kinases.

4. Cellular toxicity and selectivity of AMPs

To enter clinical use as novel pharmaceutical preparations, AMP delivery should ideally be widely therapeutic and nontoxic to mammalian cells. In theory, AMPs show selective toxicity only towards bacterial cells and cationic AMPs do not interact with the eukaryotic host cells due to their positive charge and amphiphilic nature. AMPs disrupt bacterial cell membranes and induce death of bacteria through self-assembly, by inducing conformational changes or more commonly by electrostatic or hydrophobic forces with the negatively charged lipids present on the bacterial membrane (Galdiero et al., 2013). In practice, and despite the neutral or zwitterionic lipids present on mammalian cell surface, high spiking concentrations and repeated administration required for the antimicrobial effectivity of free AMPs results in loss of selectivity, binding and disruption of mammalian cell membranes. Physicochemical features of AMPs such as charge, hydrophobicity (Edwards, Elliott, Kavanagh, Blaskovich, & Cooper, 2017) and structure are the main determinants for both their antimicrobial activity and host cellular interactions (Pirtskhalava, Vishnepolsky, Grigolava, & Managadze, 2021). Uncontrolled AMP delivery is thus associated with local and systemic cytotoxicity (Falagas & Kasiakou, 2006;Dijksteel, Ulrich, Middelkoop, & Boekema, 2021; Eckert, 2011; Hansen, Schäfer, Knappe, Seibel, & Hoffmann, 2012; Magana et al., 2020; Marr, Gooderham, & Hancock, 2006). Moreover, development of novel AMPs with wider therapeutic indexes to prevent such untargeted adverse host interactions has proven to be challenging (Aoki & Ueda, 2013). Some advances to obtain increased bacterial selectivity have, nevertheless, been made with different strategies. For example, reducing AMP

hydrophobicity helps to decrease their haemolytic toxicity (Chen et al., 2007). Increased hydrophobicity of AMPs, such as identified in the frog skin-derived AMP magainin, leads to higher affinity interactions with bacterial cell membranes and enhanced bacterial killing (Tachi, Epand, Epand, & Matsuzaki, 2002). Reduced toxicity is also achievable through such structural and chemical modifications as introduction of D-amino acids, cyclization, fluorinated amino acids and PEGylation (Matsuzaki, 2009). For example, substitution of the 15th amino acid threonine to serine in the insect-derived thanatin yields analogous S-thanatin, which demonstrates high bacterial cell selectivity and broad antibacterial activity (Wu et al., 2010). Unfortunately, results from *in vitro* toxicity testing are methodologically highly variable and often do not correlate to clinical situations (Greco et al., 2020). Nanocarrier controlled release systems have emerged promising for sustained and concentration-controlled AMP delivery methods that can circumvent the high-concentration-spikes-associated toxicity accompanying more traditional and repeated free AMP delivery methods. Examples of such systems are provided in paragraph: "*Novel nanocarrier delivery strategies with AMPs and their significance*".

5. AMPs in wound infections and their mechanisms of action

Wounds compromise the skin's barrier to the environment. Microbes are almost always present in skin wounds. Chronic, non-healing wounds are characterized by failure of the host tissue to gain control over the scene through coordination of intrinsic microbial killing mechanisms, inflammation and immune responses. In patients suffering from chronic wounds, this setting is usually further compromised by poor arteriovenous and lymphatic circulations, impaired innervation, both local and systemic defects in metabolic control and formation of drug resistant microbial biofilms (FrykbergRobert, 2015). In all their complexity, such hard-to-heal infected wounds emerge as optimal therapeutic targets for AMPs with broad-spectrum antimicrobial activities and immunomodulatory actions. Table 1 lists examples of AMPs and their antimicrobial potential as candidates for wound infection treatment.

AMPs have antimicrobial activity against microbial biofilms (Di Somma, Moretta, Canè, Cirillo, & Duilio, 2020), and the microbes less likely establish resistance to AMPs than to antibiotic drugs. This activity has mainly been attributed to the AMPs' mode of action, combined killing and antigen neutralization, independent of microbial structure dynamics (Batoni, Maisetta, Lisa Brancatisano, Esin, & Campa, 2011; Pulido, Nogués, Boix, & Torrent, 2012). Hence, the AMPs' mechanisms of action are independent of bacterial metabolic processes in contrast to antibiotics which utilise the microbes' metabolic machineries for their antimicrobial action. AMPs mainly interact with the bacterial cell membranes causing pore formation and leakage of cellular components. Classically, the activity of AMPs involves electrostatic interactions between their positive amino acid residues and negatively charged bacterial surfaces (Kumar, Kizhakkedathu, & Straus, 2018). The membrane lipid composition (anionic, cationic, or zwitterionic lipids) can determine the susceptibility of the bacteria to the action of certain antimicrobial agents (Epand & Epand, 2011). Moreover, the surface charge, amino-acid composition, peptide structure, and peptide/lipid ratio play roles in this interaction (Li et al., 2017). The AMP-bacteria interactions are illustrated in Fig. 3, and include: barrel stave, carpet model (Yang, Harroun, Weiss, Ding, & Huang, 2001), toroidal pore, and electroporation (Sengupta, Leontiadou, Mark, & Marrink, 2008). Oxidized phospholipids are high affinity targets for AMPs and they increase AMP adsorption to bacterial surfaces. The coupling of peptides and anions with subsequent efflux disrupts the membrane potential. Subsequently, AMPs can then translocate across the membrane aided by the electroporation process (Mahlapuu et al., 2016). Several models describe the AMP-bacterial membrane interactions and AMP permeation (Nguyen, Haney, & Vogel, 2011; Toke, 2005). In the barrel stave model, peptides align perpendicularly to each other to form transmembrane pore structures, in which the peptides hydrophobic regions face the membrane's lipid core while hydrophilic regions form the edges of the pore. According to the toroidal pore model, membrane insertion of peptides causes the bending of phospholipids with pores lined by both peptides and hydrophobic phospholipid groups. In the carpet model, AMP binding evokes membrane tension causing formation of micelles (Yeaman & Yount, 2003). The leakage of important metabolites and ions and respiratory inhibition then cause cell lysis (Brogden, 2005). AMPs are also translocated to the cytoplasm or bacterial protoplasm and can cause inhibition of nucleic acid DNA/RNA, disruption of protein folding and inhibition of cell wall synthesis leading to cytotoxicity and cell death (Nicolas, 2009).

Although emerging at a slower rate compared to antibiotic resistance, bacterial resistance to AMPs has also been described (Kubicek-Sutherland et al., 2016; Andersson, Hughes, & Kubicek-Sutherland, 2016). Because the antimicrobial activity of AMPs relies on membrane interactions, bacteria utilize membrane modifications as their primary resistance mechanisms to AMPs. For example, Gram-negative bacteria like *E. coli*, and *Salmonella* develop resistance through LPS modifications that alter the charge and fluidity of their membranes. Other mechanisms involving changes in membrane channels and their expression have been reported. These involve efflux pumps, membrane transporters, ion transport, and ATP binding cassette (ABC) transporters (Gunn, 2001). Bacterial resistance to AMPs can also develop through bacterial capsule or biofilm formation, secretion of proteases, and sequestering or efflux of AMPs. An intriguing mechanism involves bacterial cleavage of host cell surface structures to bind and neutralize AMPs (Band & Weiss, 2015). Increased hydrophobicity of bacterial LPS by modifying lipid chains, glucosamine, lipid phosphates or acylation (Guo et al., 1998) can increase LPS saturation and prevent the functional incorporation of AMPs for example in *Helicobacter pylori* (Tran et al., 2006). Gram-positive microorganisms like *Bacillus*, *Clostridium, Staphylococcus* and *Enterococcus spp* develop resistance against AMPs through for example membrane modifications, gene expression alterations, proteases and other inactivation procedures (Assoni et al., 2020). Bacterial resistance mechanisms to AMPs have been reviewed extensively and readers are referred to the following references (Andersson et al., 2016; Assoni et al., 2020; Gruenheid & Le Moual, 2012; Yeaman & Yount, 2003). To combat antibiotic resistance, combinations of AMPs with current antibiotics are suggested to be used. Such combinations are less inclined to cause resistance or cross-resistance development (Lewies, Du Plessis, & Wentzel, 2019).

6. Wound healing process and role of AMPs

Wound healing is a multistep biological process that progresses through four overlapping phases—hemostasis, inflammation, proliferation, and tissue remodeling—to restore integrity and to drive regeneration of the tissue (Singh, Young, & McNaught, 2017). Each phase has its own characteristic fingerprint signaling networks of cell types, cell-cell and cell-matrix interactions, and paracrine signals that contribute to and drive wound healing progression to the next phase, and ultimately to full repair (Guo & DiPietro, 2010). Cells active in the wound healing response can be divided into three subsets: 1) neutrophils, T and B cells, 2) monocytes and macrophages, and 3) non-leukocytic cells including keratinocytes, fibroblasts and endothelial cells (Mirza & Koh, 2015). Importantly, all these cells produce AMPs, which kill microbes and modulate the wound healing process. AMPs elicit varying actions that are dependent of the stage of wound healing (Fig. 4) as evidenced in *in vitro* cell migratory studies and *in vivo* healing activities in small animals (Table 2). For example, the LL-37 peptide aids wound healing by regulating cell migration (Niyonsaba et al., 2002; Yang et al., 2000), inflammation, and angiogenesis (Ramos et al., 2011).

Wound healing commences with *hemostasis* to restrict blood loss and promote the clotting process and matrix formation, also termed as the *lag phase,* which persists from days to weeks and is followed by

Table 1
The efficacy of AMPs against common and resistant wound infections.

AMP	Antimicrobial activity	Efficacy determination	Application	Reference
A3-APO	*A. baumannii*	more efficacious and less toxic against MDR Gram-negative pathogens, burn injury in mice	wound infections after burn injury	(Ostorhazi et al., 2010)
Brevinin-2Ta	*S. aureus*, *E. coli* and *C. albicans*	reduced inflammation and re-epithelialisation of *K. pneumoniae* infected rat wounds	infected wounds	(Liu et al., 2017)
Cecropin P1	*S. aureus* and combination with *P. aeruginosa*	accelerated granulation of the wound bed and marginal epithelialization	wound healing activity	(Lebedeva et al., 2017)
DGL13K	*P. aeruginosa*, *S. aureus* and MRSA	low toxicity to human red blood cells and HEK cells, no skin toxicity, killed *P. aeruginosa* in both the *G. mellonella* model and a mouse burn wound infection model	skin burn infections, topical treatment	(Gorr, Flory, & Schumacher, 2019)
DRGN-1	*P. aeruginosa* and *S.aureus*	potent antimicrobial and anti-biofilm activity, migration of keratinocyte and pathogen-directed and host-directed activities in clearing of the polymicrobial biofilm infected wounds	infected wounds	(Chung, Dean, Propst, Bishop, & van Hoek, 2017)
Epinecidin	MRSA	skin injury in mice	infected wounds	(Huang et al., 2013)
IDR-1018	*S. aureus*	porcine wound healing model	wound healing and infection control	(Steinstraesser et al., 2012)
IRIKIRIK (IK8L), IRIklrIK (IK8-2D), irikirik (IK8D)	MDR *P. aeruginosa* strains	superior antibacterial killing than selected antibiotics, membrane-lytic antimicrobial mechanism, effective in the *in vivo* infected burn wounds	burn wound infections	(Zhong et al., 2017)
K11	*A. baumannii*	early clearance of infection and wound clearance in murine excision model	infected excision wound	(Rishi et al., 2018)
LL-37	MRSA	human epidermal models	burn wounds infected with MRSA	(Haisma et al., 2014)
Nisin	*S. aureus*	rabbit full thickness burn wound	bacteria-infected chronic wound	(Qu et al., 2019)
Novispirin G10	*P. aeruginosa*	radial diffusion assay	burns or other wound infections	(Steinstraesser et al., 2002)
Pep 19-2.5	MRSA	promotes artificial wound closure in keratinocytes and *in vivo* cytosolic calcium and mitochondrial ROS e P2X7R for peptide-induced phosphorylation of ERK1/2	non-infected and *S. aureus*-infected wounds	(Pfalzgraff et al., 2018)
PMAP 36PW and Myr-36PW	*S. aureus* and *P. aeruginosa*, anti-biofilm activity against gram-negative bacteria	kill bacteria by affecting membrane permeability, promote abscess reduction and wound healing in infected mice	wound healing and bacterial infection control	(Liu et al., 2020)
Pseudin-2 and Pse-T2	MDR *P.aeruginosa*	bacterial killing by marked pore formation and permeabilization, facilitated wound closure	MDR bacterial skin strain infections	(Kang, Seo, Luchian, & Park, 2018)
RP504, RP556 and RP557	Gram-positive and Gram-negative bacteria and fungi, including recalcitrant biofilm	*in vitro* assays against fungal and bacterial infections, mammalian cell toxicity	cutaneous fungal infections	(Woodburn, Jaynes, & Clemens, 2020)
SAAP-148	MDR gram-positive and gram-negative ESKAPE pathogens, as well as isolates of *E. cloacae*, *E. coli*, and *K. pneumoniae*	*ex vivo* infection control in human skin and i*n vivo* murine skin	biofilm associated skin infections	(de Breij et al., 2018)
linSB056-1, dendrimeric derivative	*P. aeruginosa*	reduction of *P. aeruginosa* biofilm formation tested in *in vitro* wound model	antibiofilm properties in chronic wound infections	(Grassi et al., 2019)
SHAP1	*S. aureus*	wound closure activity inducing HaCaT cell migration, did not affect the cell viability of human keratinocytes	infected wound, topical treatment	(Kim et al., 2014)
TC19	MDR strains of ESKAPE panel, viable MRSA and MDR *A. baumannii*	low cytotoxicity in human fibroblasts and effective in mouse skin wound infections model	rapid killing of MDR bacterial stains, neutralised proinflammatory, chemotaxis to neutrophils, skin infection control, topical treatment	(Riool et al., 2020)
TCP 25	*S. aureus* and *P. aeruginosa*	reduction of LPS-induced local inflammatory response in porcine wound models, peptide prevented infection with *S. aureus* and reduced proinflammatory cytokines	bacterial infection and associated inflammation, topical treatment	(Puthia et al., 2020)
Temporin A	MRSA	significant reduction of bacterial load, granulation tissue formation and collagen deposition	wound infection management and repair process	(Simonetti et al., 2008)
Tilapia Piscidin 4 (TP4)	MRSA	proliferation of a keratinocyte cell line (HaCaT) and fibroblast cell line (Hs-68) combat infections in mice through control of growth factors	antimicrobial and wound closure activities	(Huang, Chan, Wu, & Chen, 2015)
WRL3	MRSA	rapid bactericidal activity against the proliferation of MRSA in wound infections, synergistic effects with antibiotics	MRSA burn wound infections and wound healing	(Ma et al., 2017)

A3, APO proline-rich antibacterial peptide; DGL13K, D-amino acid version of the peptide GL13K; DRGN-1, synthetic peptide inspired by a peptide identified from Komodo dragon; ERK, protein-serine/threonine kinases; ESKAPE, an acronym comprising the scientific names of six highly virulent and antibiotic resistant bacterial pathogens including: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp; HaCaT, human keratinocyte cells; HEK cells, human embryonic kidney cells; Hs-68, human fibroblast cells; IDR-1018, innate defense regulator *1018;* IRIKIRIK (IK8L), IRIklrIK (IK8-2D), irikirik (IK8D), synthetic β-sheet folding peptides; LPS, lipopolysaccharide; K11, Lysine homopeptide with odd number of residues; LL-37, 37-amino-acid-long cathelicidin C-terminal peptide; MDR, multidrug resistance; MRSA, methicillin resistant *Staphylococcus aureus*; Myr-36PW,; WRL3, engineered amphipathic peptide; Pep 19-2.5, synthetic peptide; PMAP 36PW, porcine antimicrobial peptide analog; RP504, RP556 and RP557, synthetic designed AMPs; Pse-T2, Pseudin-2 analog; Pseudin-2, peptide isolated from the frog *Pseudis paradoxa*; ROS, reactive oxygen species; SAAP-148, synthetic peptide which parent peptide is LL-37; linSB056-1, semi-synthetic peptide; SHAP1, 19-amino-acid designer peptide; TC19, Thrombocidin-1-derived synthetic peptide; TCP25, thrombin C-terminal peptide; Temporin A, frog-derived small antimicrobial peptide; TP4, Tilapia piscidin 4, 23 amino acid pore-forming peptide.

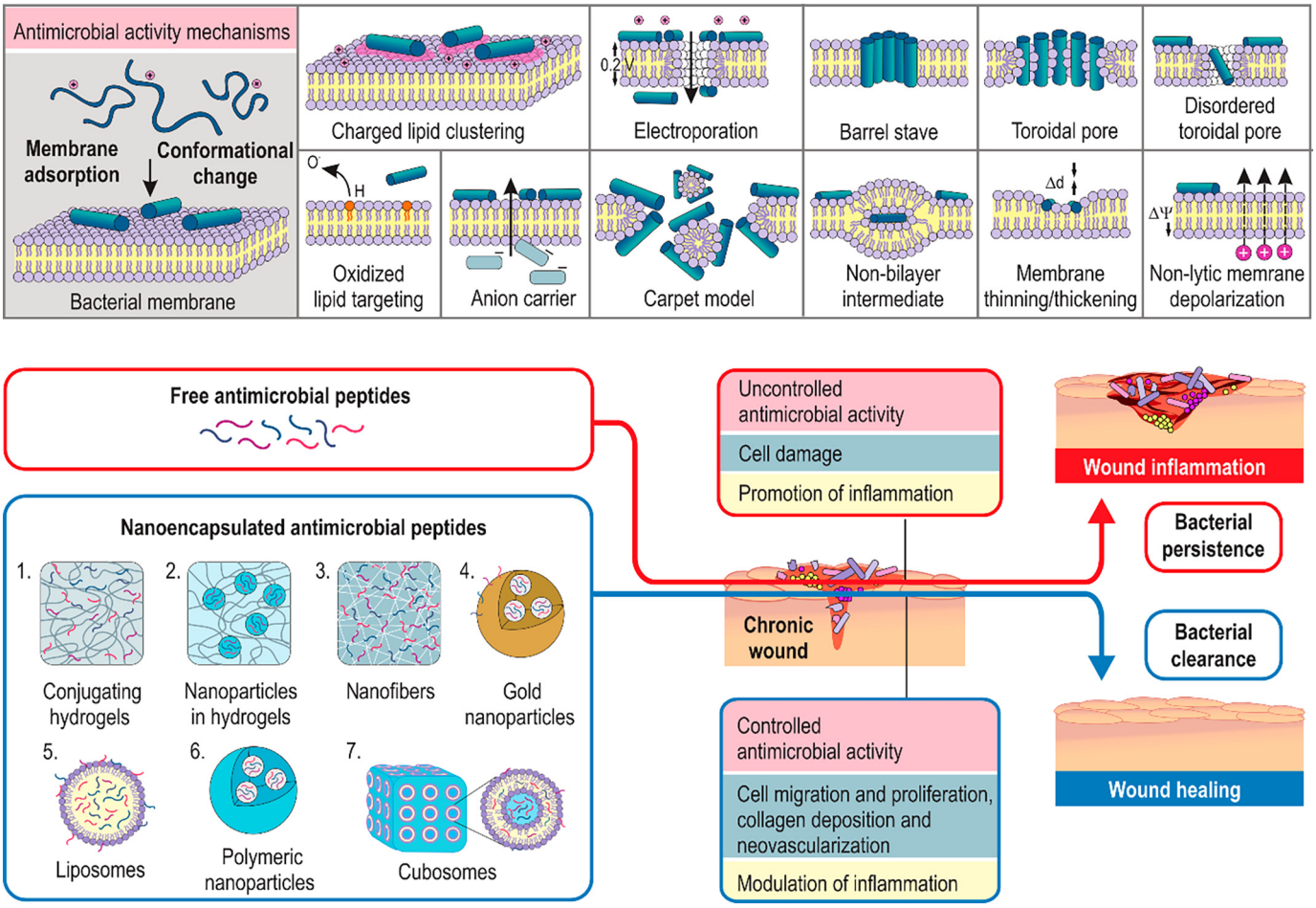

Fig. 3. Schematics showing the interaction at the cytoplasmic membrane upon antimicrobial peptides (AMPs) adsorption important for their antimicrobial activity and the action mechanisms of free AMPs *vs* nanoencapsulated AMPs during wound infection treatment. Different models explain the interactions on the membrane and AMPs antimicrobial activity mechanisms (upper panel). Antimicrobial activity and wound healing properties of free AMPs and nanoencapsulated AMPs using different carrier systems: 1. Conjugating hydrogels, 2. Nanoparticles in hydrogels, 3. Nanofibers, 4. Gold nanoparticles, 5. Liposomes, 6. Polymeric nanoparticles, 7. Cubosomes (lower panel).

an inflammatory process in an overlapping manner. Hemostasis is characterized by platelet activation, vasoconstriction, and activation of the coagulation cascade. Innate immune mechanisms, such as complement activation, that interact with these processes further contribute to the hemostasis phase. Many AMPs are formed from protein fragments, including C3a, which have antimicrobial properties (Pasupuleti et al., 2007). Further, these processes lead to priming of the inflammatory phase by increasing the vascular permeability and penetration of inflammatory mediators to the wound site, promoting the development of nitric oxide, adenosine, and other vasoactive metabolites, and causing histamine release from mast cells and vasodilation (Sorg, Tilkorn, Hager, Hauser, & Mirastschijski, 2017).

The inflammatory phase comprises the events of activation and migration of neutrophils and monocytes releasing growth factors and cytokines, such as TNFα, IL6, -8 and vascular endothelial growth factors (VEGF). This is accompanied by macrophages engulfing pathogens, dying neutrophils and debris (Robson, Steed, & Franz, 2001). During inflammation, neutrophils are the most essential cells that produce AMPs (Borregaard & Cowland, 1997). Defensins, present in the azurophilic granules, and cathelicidins are examples of these AMPs. The ability of LL-37 to dampen and modulate inflammation through the NFκB-TNF-α axis (Alalwani et al., 2010) helps the inflammation from going unchecked. In chronic wounds with delayed healing, an active and persistent inflammatory process is associated with defects in the production of LL-37 (Heilborn et al., 2003). LL-37 inhibits keratinocyte apoptosis and increases VEGF production through cellular inhibitor of apoptosis protein 2 (cIAP-2) and hypoxia- inducible factor-1α signaling (Rodríguez-Martínez, Cancino-Diaz, Vargas-Zuñiga, & Cancino-Diaz, 2008). Moreover, LL-37 stimulates keratinocyte migration via EGFR transactivation (Tokumaru et al., 2005). PR-39 (proline and arginine-rich peptide) is a potent angiogenic regulator that inhibits ubiquitin proteasome-dependent degradation of hypoxia- inducible factor-1α protein contributing to accelerated growth of vascular structures in mice (Li et al., 2000). PR-39 can limit cell damage during inflammation by limiting the NADPH oxidase-induced superoxide anion O^{2-} production (Shi, Ross, Leto, & Blecha, 1996).

The proliferation stage is aimed at re-epithelization and production of cytokines such as interferons (IFNs), transforming growth factor β (TGF-β), collagen deposition, fibronectin, and other essential substances required by fibroblasts for wound healing and for the production of a new connective tissue matrix (Baum & Arpey, 2005). During the proliferation stage of wound healing, most AMPs, such as hBD2, hBD3, RNase7, and psoriasin, are produced by epidermal keratinocytes (Roupé et al., 2010). For example, the expression of hBD2 and hBD3 is induced in human skin wounds by EGFR activation (Roupé et al., 2010). The subsequent phosphorylation of signal transducer and activator of transcription (STAT) proteins then stimulates cytokine production, migration, and proliferation of keratinocytes (Niyonsaba et al., 2007). TGF-β1 stimulates angiogenesis, fibroblast differentiation, matrix deposition and regulates production of growth factors and

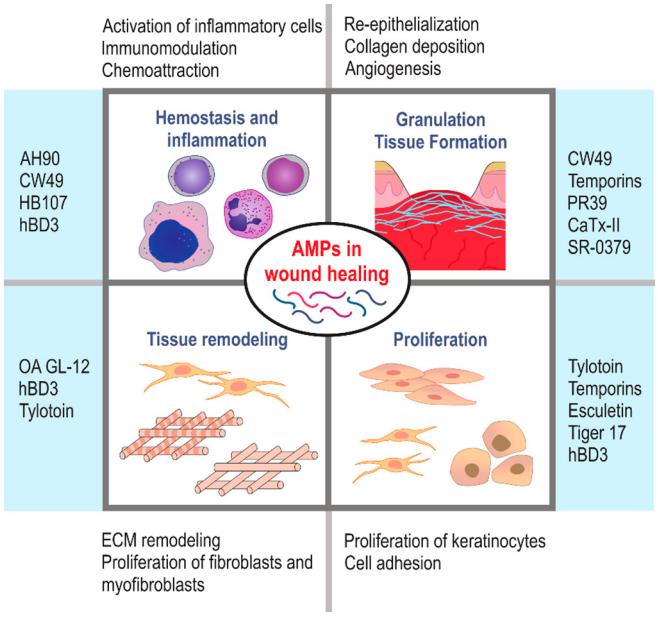

Fig. 4. The involvement of antimicrobial peptides (AMPs) in the wound healing process at various stages. Key: AH90, wound healing promoting peptide (amino acid sequence ATAWDFGPHGLLPIRPIRIRPLCG) from frog skin of *Odorrana grahami*; AMPs, antimicrobial peptides; CaTx-II, *Crotalus adamanteus* toxin-II; CW49, wound-healing promoting peptide (amino acid sequence APFRMGICTTN) from frog skin of *Odorrana grahami*; ECM, extracellular matrix; HB107, peptide derived from the antimicrobial cecropin B; hBD3, human β-defensin-3; OA GL-12, *Odorrana andersonii*, GL: two initial amino acids, 12: peptide length; PR39, porcine cathelicidin; SR-0379, modified metabolite peptide; Tiger 17, wound healing promoting small peptide (c [WCKPKPKPRCH-NH_2])

cytokines such as TNF-α and IL-6 in the process of wounding (Ahmed & Ikram, 2016; Peng et al., 2011). The production of TNF-α marks the activation of macrophages (Ashcroft et al., 2012), contributes to keratinocyte migration (Banno, Gazel, & Blumenberg, 2004), modifies their proliferative activity (Detmar & Orfanos, 1990), stimulates fibroblast proliferation (Battegay, Raines, Colbert, & Ross, 1995) and affects angiogenesis in concentration dependent manner (Fajardo, Kwan, Kowalski, Prionas, & Allison, 1992). IL-6 stimulates angiogenesis, keratinocytes, as well as fibroblast proliferation (Johnson, Stevenson, Prêle, Fear, & Wood, 2020).

The re-epithelialization process involves keratinocyte proliferation and migration. Growth factors like IGF1 and TGF-α induce production of AMPs in human keratinocytes (Sørensen et al., 2003). For example, AMPs such as LL-37, hBD3, neutrophil gelatinase-associated lipocalin, and secretory leukocyte protease inhibitor are produced throughout the re-epithelization stage (Heilborn et al., 2003; Sørensen et al., 2003). In human skin, LL-37 can induce re-epithelization after wounding (Heilborn et al., 2003). LL-37 directly stimulates endothelial cells initiating enhanced proliferation and growth of vessel structures facilitated by formyl peptide receptor-like 1 (FPRL1) (Koczulla et al., 2003). It can control tissue regeneration and neovascularization by inducing the production of growth factors, especially VEGF (Kittaka et al., 2013; Rodríguez-Martínez et al., 2008). Moreover, LL-37 contributes to angiogenesis through COX-1-dependent prostaglandin E2 receptor EP3 (PGE2-EP3) signaling in endothelial cells (Salvado, Di Gennaro, Lindbom, Agerberth, & Haeggström, 2013). The absence of cathelicidin-related antimicrobial peptide (CRAMP) in mice similar to LL-37 in humans, delays the vascularization of wounds (Koczulla et al., 2003). In addition to suppressing the production of proinflammatory cytokines, LL-37 may also contribute to inhibition of fibroblast and myofibroblast activities (Liu et al., 2016) to orchestrate the resolving of inflammation (Inomata, Into, & Murakami, 2010), and control the production of connective tissue collagens I and III.

7. Novel nanocarrier delivery strategies with AMPs and their significance

AMPs are effective disruptors of biological membranes. Thus, it is not surprising that at high concentrations their cytotoxic activity can also be directed towards host cells, thereby limiting their therapeutic value. Furthermore, as peptides, AMPs undergo relatively rapid enzymatic degradation. This contributes to an unfavorable pharmacokinetic profile as well as to issues with stability (Bradshaw, 2003). Attempts to solve these concerns have been devised through the rational design of chemical modifications aimed at enhancing the peptides' antimicrobial or wound-healing activity while reducing their cytotoxicity (Torres, Sothiselvam, Lu, & de la Fuente-Nunez, 2019). An alternative or even additive approach is the utilization of nanotechnology for the delivery of AMPs to fight drug-resistant infections. Nanotechnology-based therapeutics enhance the stability and efficacy of AMPs and reduce their toxicity to host tissue cells (Pal et al., 2019). Nanomaterial-based antibiotic delivery, termed as *nano antibiotics*, can provide improved solubility, stability, bioavailability, and sustained activity, as well as increased internalization with reduced dose and side effects (Huh & Kwon, 2011). The use of suitable nanocarriers to encapsulate the AMPs for improving their therapeutic potential is called *nanoencapsulation* (Yadav, Kumari, & Yadav, 2011). The encapsulation of AMPs in a nanomaterial holds great potential due to their small size, high surface area, targeting ability, functionalization, specificity, lower toxicity, and higher efficacy (Noorbatcha, Jaswir, & Ahmad, 2017). In addition, entrapment of AMPs in nanocarriers can prevent the inherent problems of AMPs delivery, like proteolytic degradation and undesirable membrane interactions (Brandelli, 2012). AMP-conjugated nanoparticles (NPs) offer enhanced antimicrobial activity (Rai et al., 2016), lower toxicity and provide higher stability compared to AMPs alone (Maleki et al., 2016). Nanoencapsulation of AMPs is achievable by either active or passive targeting-based systems. The passive delivery of AMPs involves encapsulation of peptides into nanocarriers without modifications or change in surface chemistry. Encapsulation of AMPs in passive nanocarriers improves their stability, shelf-life (da Silva Malheiros, Sant'Anna, Utpott, & Brandelli, 2012), sustained release (Prombutara, Kulwatthanasal, Supaka, Sramala, & Chareonpornwattana, 2012) and activity (Severino et al., 2017) as well as limits undesired side-effects (Yousry, Elkheshen, El-Laithy, Essam, & Fahmy, 2017). The active delivery systems are based on the addition of a target-specific high-affinity ligand or functionalization agent that guides the AMPs specifically to their desired site of activity: the surface of the microbe targeted for killing. Alternatively, functionalization can be aimed against structures presented by infected and activated cells. Nanocarriers with such surface ligands interact specifically with the infected cells, provide higher AMPs- or drug-loads and improved efficacy, and prevent off-site side effects (Biswaro, da Costa Sousa, Rezende, Dias, & Franco, 2018). The commonly used techniques for designing the biomaterial for encapsulation of AMPs can be categorized into inorganic materials, polymeric carriers, and lipid-based approaches (Fig. 3). Examples of nanocarriers encapsulating AMPs explored for this purpose are listed in Table 3.

7.1. Gold nanoparticles (AuNPs)

Encapsulation of AMPs into NPs is the most often used approach for enhancing stability and preventing toxicity associated with high AMP concentrations. Inorganic NPs, such as gold NPs (AuNPs), are an attractive approach for AMPs delivery. They are highly biocompatible and can be easily functionalized using conjugation chemistry. Noncovalent AMP conjugation with AuNPs is achieved through self-assembly of the peptide through one-step synthesis. Using cysteine as the reducing and capping agent to achieve proper orientation of AMPs, the hydrophilic AMP shell assembles around the Au core (Chen et al., 2015; Rai et al., 2016). Self-assembled AMPs on the NP surface synergistically increase AMP affinity and specificity at the bacterial targets' interacting sites through

Table 2
In vitro and *in vivo* evidence of AMPs efficacy in the wound healing process.

Peptide	Source	*In vitro* activity	Mechanism	*In vivo* wound healing effects	Reference
Omiganan	cytoplasmic granules of bovine neutrophils		limited recruitment of neutrophils, lymphocytes, histiocytes	improved re-epithelialization, neovascularization, inflammation, and collagen depletion	(Lorenzi et al., 2017)
Nisin A	gram-positive bacteria	migration of HaCaT and HUVECs, decrease in TNFα, IL6, IL8	immunomodulatory effect	improved re-epithelization in *ex vivo* porcine wound healing and survival from Gram-positive bacteria *G. mellonella*	(Mouritzen et al., 2019)
Myxinidin	hagfish, fish, animals	antimicrobial activity *S.aureus*, *A.baumannii*, and *P. aeruginosa*	suppressed phosphorylation of JNK, STAT3, and EGFR pathway	decreased IL6, IL8, TNFα, enhanced wound healing in animals and re-epithelization by EGFR	(Han et al., 2017)
Myticin C	mollusk, invertebrates, animals	modulation of migration genes myosin, transgelin, calponin, and motility of hemocytes	gene encoding ECM proteins HSPG2 and proteoglycan	larvae zebrafish models, tail-fin amputation, chemotactic, and healing activity	(Rey-Campos et al., 2020)
Coprisin	insects, arthropods, invertebrates, animals		decreased phosphorylated-Smad2/3 levels, TGFβ pathway	wound closure neovascularisation and promotion of re-epithelization	(Lee et al., 2013)
Temporin A and B	frogs, amphibians, animals	wound healing in HaCaT cells	EGFR signaling pathway	----	(Di Grazia, Luca, Li-av, Shai, & Mangoni, 2014)
SHAP1	synthetic	inducing HaCaT cell migration	EGFR pathway	*S. aureus* infection and improved wound healing in a murine full-thickness excision model	(Lee et al., 2014)
Tilapia Piscidin 4 (TP4)	fish, animals *Nile tilapia* (*Oreochromis niloticus*)	cell proliferation activated collagen I, III, gene KGF in Hs-68 cells inducing production by HaCaT cells		antimicrobial, EGF, TGF, and vascular VEGF activity wound closure in mice	(Huang et al., 2015)
AH90	frog skin, *Odorrana grahami*	wound closure activity on HaCaT cells	NF-β and c-Jun NH2-terminal kinase MAPK signaling. Smad2/3 activation via the TGFβ mechanism	wound healing in mice, increased TGFβ secretion, peptide-induced granulation contraction	(Liu et al., 2014)
CW49	frog skin of *Odorrana grahami*	induces angiogenesis *in vitro* in HUVECs	angiogenic proteins (HIF-1, eNOS and iNOS) and inhibition of TNFα and IL6	accelerated wound healing by angiogenesis and prevention of inflammatory effect, affect granulation and epithelium thickness	(Liu et al., 2014)
Tiger 17	synthetic peptide	proliferation in HaCaT and HSFs cells	MAPK signaling pathway	macrophages induction, migration, and proliferation of keratinocytes and fibroblasts, increased re-epithelization, granulation tissue remodeling, TGFβ1, and IL6	(Tang et al., 2014)
HB107	derived from antimicrobial cecropin B	IL8 secretion from cultured HMVECs	leukocyte phagocytosis and cytokine production	murine wound healing, full-thickness skin decreases in wound diameter compared to vehicle control	(Lee et al., 2004)
K11 (Melittin, Cecropin A1 and Magainin 2)	hybrid and synthesized	cell lysis, leakage of cytoplasm	antimicrobial and antioxidant activity	*Acinetobacter baumannii* murine model complete clearance of infection, wound enclosure, increased antioxidant activity, wound remodeling, re-epithelization	(Rishi et al., 2018)
R 39	pigs, mammals; animals	activity against *Bacillus globigii* and *Enterococcus faecalis*.	induced ATP leakage and loss of membrane, lytic and induce IL8 in porcine macrophages, TNFα production	----	(Veldhuizen et al., 2014)
hBD3	primates, mammals, animals			transgene expression, bacterial infection, re-epithelialization, wound contraction, wound fluid production, and blood vessel formation	(Hirsch et al., 2009)
Lucifensins	insect defensins, hemolymph of the fly larva			inhibition of neutrophils, fibroblast migration	(Čeřovský & Bém, 2014)
CaTx-II	venom of *Crotalusadamanteus*	bactericidal effects on *S.aureus*, *Burkholderia*, pseudomallei and Enterobacter aerogenes	NF-kβ activation, IL1β suppression	treated mice wound closure in 16 days, enhanced collagen synthesis, neovascularization.	(Samy et al., 2014)

AG30/5C, angiogenic peptide; AKT, protein kinase B; ATP, adenosine triphosphate; CaTx-II, Crotalus adamanteus toxin-II; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular-signal-regulated kinase; HaCaT, human keratinocyte cells; hBD3, human β-defensin-3; HB107, peptide derived from the antimicrobial cecropin B; HIF-1, hypoxia-inducible factors; HMVEC, human microvascular endothelial cells; HSPG2, heparan sulphate proteoglycan; HSF, human skin *fibroblasts*; Hs-68, fibroblast cell line; HUVECs, human umbilical vein endothelial cells; IGFβ, insulin-like growth factor beta; IL-6,8, interleukin 6 and 8; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; KGF, keratinocyte growth factor; MAPK, mitogen-activated protein kinase; NF-kβ, nuclear factor kappa-light-chain-enhancer of activated B cells; NHDFs, primary normal human dermal fibroblasts; OA-GL12, *Odorrana andersonii*, GL: two initial amino acids, 12: peptide length; PI3K, phosphoinositide-3-kinase; PI3kinase-Akt-mTOR, phosphoinositide3-*kinase* protein *kinase* B-mammalian target of rapamycin; PR-39, porcine cathelicidin; RAW 264.7, macrophage cell line; SHAP1, 19-amino-acid designer peptide; Smad2/Smad3, small mothers against Decapentaplegic; SR-0379, modified metabolite peptide; STAT3, signal transducer and activator of transcription 3; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

concentrated multiple similar binding interactions (Chen et al., 2015). Moreover, this leads to high local AMPs concentrations on the bacterial surface. Covalent conjugation of AMPs, on the other hand, is mostly achievable by long ligand exchange reactions or hydrophobic interactions between peptide side chains such as lysine and the Au surface. The interactions between the AMP lysine groups and Au generate a randomly folded AMP cover onto the Au surface. The conjugation of AMPs to AuNPs, proposed as one pot synthesis (Wadhwani, Heidenreich, Podeyn, Bürck, & Ulrich, 2017), increases AMP stability against trypsin digestion while allowing AMP conversion into active conformations. Therefore, covalently conjugated AMPs to AuNPs can induce strong bactericidal effects (create more and/or larger holes) and

Table 3
Examples of nanocarriers used for antimicrobial peptides (AMPs) delivery for wound-healing application

Polymers and formulation	Method of incorporation	Peptide	Characterization techniques	*In vitro or In vivo* activity	Antimicrobial activity	Reference
Polyvinyl alcohol nanofibers	electrospinning	A3-APO	morphology, diameter/size, SEM temperature effect	*in vivo* antimicrobial, skin abrasion infection and tissue analysis	*MDR A. baumannii ESBL E. coli, MBL P. aeruginosa*	(Sebe et al., 2016)
Chondroitin sulfate-based nanogel	ring-opening polymerization	Nisin	FTIR, NMR, cloud point, swelling, morphology, loading, and entrapment	cytotoxicity studies on HDFCs	*S. aureus* and *E. coli*	(Ghaeini-Hesaroeiye, Boddohi, & Vasheghani-Farahani, 2020)
CHNPs	ionotropic gelation	Temporin B	particle size, zeta potential, loading capacity, and release kinetics	cytotoxicity, bactericidal assays	Strains of *S. epidermidis*	(Piras et al., 2015)
GelMA and MeTro	light-induced crosslinking	Tet213	porosity, degradability, swellability, mechanical, and adhesive properties	biocompatibility and biodegradation *in vivo*	MRSA and Gram-negative *E. coli*	(Annabi et al., 2017)
AuNPs	reduction by NaBH4, mixing LL-37, and reacting pDNA containing VEGF	LL-37	*in vitro* transfection, cytotoxicity, cellular uptake and intracellular distribution	*in vitro* antibacterial and *in vivo* wound healing	MRSA	(Wang et al., 2018)
Collagen films	mixing and pouring	Pexiganan	cytotoxicity binding studies, swelling and in vitro release studies	*in vitro* and *in vivo* antibacterial activity	*P.aeruginosa, S. aureus*	(Gopinath, Kumar, Selvaraj, & Jayakumar, 2005)
NLCs	modified double (w/o/w) emulsion technique	Nisin Z	size, zeta potential, TEM analysis	MIC of antibiotics and AMPs, toxicity, MTT and LDH assay	*S.aureus, S.epidermidis and E. coli*	(Lewies, Wentzel, Jordaan, Bezuidenhout, & Du Plessis, 2017)
Niosomes	FDEL method	Gallidermin	vascular size, zeta potential, entrapment efficiency	in vitro antibacterial assays, transdermal absorption	*P. acnes, S.aureus*	(Manosroi et al., 2010)
Photoluminescent gold nanodots	self-assembly	SFT, 1-DT	fluorescence staining and TEM	*in vitro* cytotoxicity, hemolysis, *in vivo* wound healing	*S. aureus, S. enteritidis*, MRSA, *E. coli*, *P. vulgaris*, MRSA-infected wound healing	(Chen et al., 2015)
Hydroxypropyl cellulose gel	incorporation into gel	PX150	luminescence measurements *in vivo* toxicity and local tolerance	*P. aeruginosa in vitro* and *in vivo* infected burn wound model in mouse	*P.aeruginosa*	(Björn et al., 2015)

A3-APO, proline rich peptide; AuNPs, gold nanoparticles; CHNPs, chitosan nanoparticles FDEL, freeze-dried empty liposomes method; FTIR, Fourier transform infrared spectroscopy; GelMA, gelatin methacrylamide; HDFCs, human dermis fibroblasts cells; HDF, human dermal fibroblasts; LL-37, 37-amino-acid-long C-terminal human cathelicidin peptide; LDH, lactate dehydrogenase; NLCs, nanostructured lipid carriers; NMR, nuclear magnetic resonance; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MDR, multidrug resistance; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MRSA, methicillin-resistant *Staphylococcus aureus*; MeTro, methacrylic anhydride modified tropoelastin;$NaBH_4$, sodium borohydride; SEM, scanning electron microscopy; Tet 213, peptide with amino acid sequence: KRWWKWWRRC; PX-150, synthetic antimicrobial peptide; TEM, transmission electron microscopy; SFT, surfactin; 1-DT, 1-dodecanethiol; VEGF, vascular endothelial growth factor.

massive membrane damage even in antibiotic-resistant bacteria. AMP-Au-NPs may also enter the bacteria and induce DNA breaks or ROS-induced damage (Zheng et al., 2019). The combination of AMPs with antimicrobial NPs can produce synergy against bacterial resistance (Allahverdiyev, Kon, Abamor, Bagirova, & Rafailovich, 2011). AMP-conjugated AuNPs (AMP-AuNPs) have broad-spectrum efficacy in treating infections caused by Gram-positive or -negative bacteria. AMP-AuNPs have shown reduced toxicity against mammalian cells and better activity against multi-resistant pathogens (Li et al., 2014). Together with reduction synthesis techniques promoting the inherent antimicrobial activity of AuNPs, tailoring their sizes, shapes, and chemistries to enhance their targeting have made AuNPs an invaluable tool in material and biomedical sciences (Tao, 2018). During the wound healing process, the expression of extracellular matrix collagen and its different types are key determinants of outcome. AuNPs have been shown to promote wound healing by regulating collagen synthesis and degradation (Volkova, Yukhta, Pavlovich, & Goltsev, 2016), by exerting anti-inflammatory and angiogenic properties through reduction in proinflammatory cytokines interleukin IL6, 12, TNFα, and increased production of VEGF and bFGF during the angiogenesis step (Pivodová, Franková, Galandáková, & Ulrichová, 2015), by antioxidant actions (Lau et al., 2017; Leu et al., 2012) and by increasing keratinocyte proliferation (Lu et al., 2010). The typical wound-healing process generates several inflammatory mediators, including ROS. Oxidative stress is directly related to high ROS production or impaired detoxification (Leu et al., 2012) resulting in damage to the metabolic machinery (DNA, RNA, protein, cellular, metabolic functions) and impaired wound healing (Cano Sanchez, Lancel, Boulanger, & Neviere, 2018). The topical application of AuNPs also possesses antioxidant and anti-inflammatory properties which reduce oxidative stress during the wound healing process (Vijayakumar, Samal, Mohanty, & Nayak, 2019).

Comune et al., 2017 studied the wound healing and regeneration effects of soluble and AuNPs-conjugated LL-37. The process of conjugation involved a one-step synthesis process using Au as the core and the hydrophilic cationic peptide LL-37 as the shell. In this process, the peptide acts as a capping agent affecting the kinetics of formation due to complexation of Au ions with peptides (Fig. 5A). Both soluble and immobilized peptides are internalized by keratinocytes and accumulated in the EEA-1 (early endosome protein), RAB-7 (protein belonging to small molecular weight GTPase associated with late endosomes), and EGFR positive endo-lysosomal compartments (Fig. 5B). The EGFR antagonist, but not the FPRL-1 antagonist, considerably decreased the migration of keratinocytes upon treatment with LL-37 peptide/ LL-37 AuNPs suggesting the role of EGFR transactivation in controlling migration of keratinocytes. The *in vivo* studies in mice model resulted in enhanced wound healing with LL-37 AuNPs (wound closure in10 days) more than the peptide alone (70% wound closure in 10 days). LL-37 AuNPs have higher collagen and IL6 expression and lower MPO activity than LL-37, indicating that they have greater anti-inflammatory properties than immobilized peptides. The faster wound re-epithelization and connective tissue deposition with LL-37 AuNPs as compared to pure LL-37 accounts for rapid wound healing (Fig. 5C) (Comune et al., 2017).

PEGylation of peptides can protect against degradation by enzymes, immunogenic, antigenic epitopes, and can control uptake by the reticuloendothelial system (Roberts, Bentley, & Harris, 2002). Another intriguing technique for treating bacterial infection and wound healing

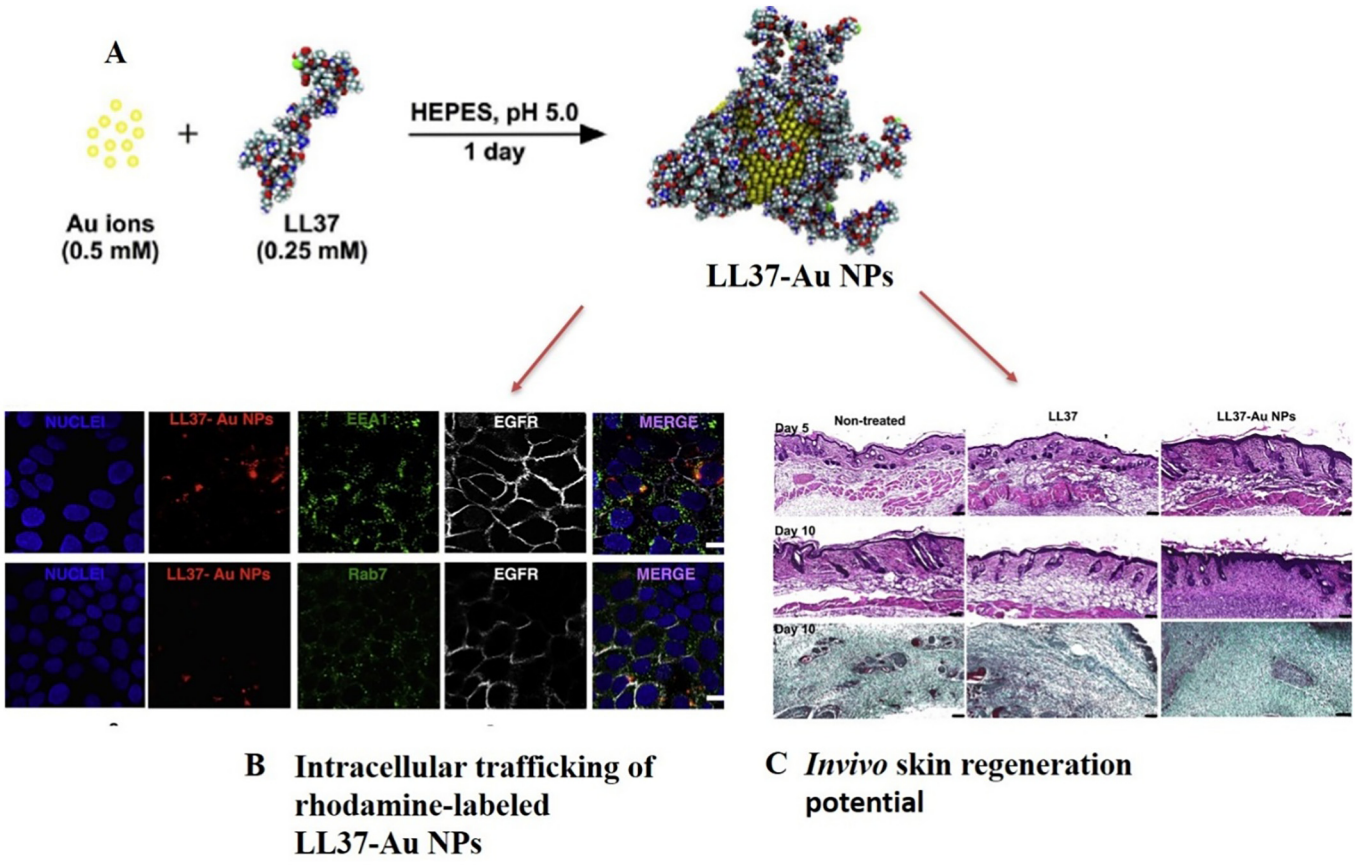

Fig. 5. A. The process of LL37-AuNP synthesis B. Intracellular trafficking of LL37-AuNPs in keratinocytes. Before confocal microscopy analysis, keratinocytes were exposed to rhodamine-labeled LL-37-AuNPs, washed, and fixed. C. Histological analysis Key: AuNPs, gold nanoparticles; HEPES, (4-(2-hydroxyethyl)-1piperazineethanesulfonic acid; LL37AuNPs, cathelicidin antimicrobial peptide encapsulated in gold nanoparticles; MPO, myeloperoxidase; IL6, Interleukin6; FPRL1 antag, formyl peptide like receptor-1 antagonist; WRW4, formyl peptide receptor like 1; EGFR antag, epidermal growth factor receptor antagonist. Reprinted with permission from ref. (Comune et al., 2017). Copyright 2017, Elsevier B.V.

is covalent binding of the peptide to AuNPs through the polyethylene glycol (PEG) linker [168]. The frog skin AMP Esc (1–21) is a derivative of esculentin-1a, and it is extremely effective against *P. aeruginosa*. The PEG-coated AuNPs conjugated to this peptide by EDC/NHS conjugation (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride)/N-hydroxysuccinimide were tested for antibacterial activity against *P. aeruginosa* and biofilm formation. This nanoassembly was more active than the free peptide against *P. aeruginosa* and resulted in higher killing activity than the free peptide with approximately 12-fold lower minimum bactericidal concentration. The improved antibacterial activity of Esc (1–21)-conjugated PEG-coated AuNPs resulted from concentrated amounts of the conjugated peptide on the bacterial surface and enhanced peptide half-life due to low accessibility to bacterial proteases and degradation. The better wound healing action in this assembly was attributed to higher resistance of peptide to enzymatic degradation, enhanced antimicrobial action by perturbation of bacterial cell membrane, and increased cell migration (Casciaro et al., 2017).

7.2. Polymeric carriers and formulations

The common natural polymers such as chitosan (CH), silk, collagen, and synthetic polymers polycaprolactone (PCL) and polylactic-co-glycolic acid (PLGA) are nontoxic, non-immunogenic, and biodegradable polymers that help in controlled release, stabilization, and toxicity reduction of AMPs (McClements, 2018). The encapsulation and release of AMPs from these polymeric carriers depend greatly on AMP and polymeric NP characteristics such as their molecular weight, structure, hydrophobicity, charge, and crystallinity as well as the dispersity index of polymeric NPs (Kamaly, Yameen, Wu, & Farokhzad, 2016).

7.2.1. Chitosan nanoparticles (CHNPs)

CH is a linear chain polysaccharide obtained from the shrimp exoskeleton after alkaline deacetylation of chitin. The polymer contains randomly distributed chains of β-linked D-glucosamine and N-acetyl-D-glucosamine (Elieh-Ali-Komi & Hamblin, 2016). CH-based biomaterials are popular in tissue engineering due to their good biocompatibility and biodegradability (Sultankulov, Berillo, Sultankulova, Tokay, & Saparov, 2019). CH is extensively used in many wound dressings and formulations currently on the market as scaffolds, bandages, sponges, matrices containing antibiotics or antimicrobials for wound healing and infection control (Dai, Tanaka, Huang, & Hamblin, 2011). The polymer provides a vast number possibilities as a delivery carrier in the form of NPs, microcapsules, gels or films for wound healing purposes together with antimicrobial action (Dai et al., 2011). CH-polymers have hemostatic activities depending on their molecular weight and deacetylation (Hu et al., 2018). The wound healing properties of CH can be enhanced by changing their N-acetylation. The presence of N-acetyl groups on CH activates both resident tissue and infiltrated cells leading to increased IL8 secretion, and forming an innate immune response-boosting loop that stimulates large numbers of neutrophils to migrate and infiltrate the wound. Reducing these groups can improve CH's properties as a biomatrix (Park, Gabrielson, Pack, Jamison, & Johnson, 2009). CH also fosters the production of growth factors TGF β1 and PDGF from macrophages, and accelerates ECM production (Ueno et al., 2001). CH affects the proliferation of keratinocytes and fibroblasts in skin defense depending on its physicochemical and functional properties like polymer molecular weight, deacetylation and functional groups (Howling et al., 2001). CH's antimicrobial action and capability of providing controlled release of drugs at the site of

infections have kept it in the limelight among polymers for a long time. Its antimicrobial action is attributed to a positive charge interacting with the negative charge on the bacterial membrane affecting bacterial permeability and causing the leakage of important intracellular components from microbes (Je & Kim, 2006). CH and its derivative polymers hold broad-spectrum antibacterial and anti-biofilm activity (Y. Tan, Han, Ma, & Yu, 2011).

CH nanoparticles (CHNPs) can provide sustained release to prolong and enhance the antibacterial activities of AMPs. The carboxymethyl derivative of naturally occurring CH, carboxymethyl chitosan (CMCH), can be used as a carrier for peptide delivery. CMCH improves the solubility of CH in aqueous media without the use of the acidic environment, exposing the active chains to pH while preserving biocompatibility, biodegradability, and other polymer properties (van der Lubben, Verhoef, Borchard, & Junginger, 2001).

OH30, a naturally occurring cationic peptide from the king cobra, belongs to the cathelicidin family. The peptide has potent antibacterial (Zhang et al., 2010; Zhao et al., 2018) selective immunomodulatory actions (Li et al., 2013), and low cytotoxicity to eukaryotic cells (Li, Lee, & Zhang, 2012). Sun and colleagues explored carboxymethyl chitosan CMCHNPs formulated by the ion gelation technique to encapsulate the OH30 peptide and evaluated its wound-healing activity (Sun et al., 2018). The authors demonstrated sustained release of OH30 from the NPs and showed improved antimicrobial activity against *E. coli*. Treatment with CMCH-NPs encapsulating OH30 resulted in improved keratinocyte migration and accelerated wound healing compared to the native peptide. The CMCS-OH30 NPs induced a higher wound closure rate because of increased re-epithelization and neovascularization and decreased production of proinflammatory cytokines.

7.2.2. Poly (lactic-co-glycolic acid) nanoparticles (PLGA NPs)

PLGA is an FDA-approved and widely used polymer with several applications in drug delivery. The popularity of PLGA lies in its biodegradability and compatibility for sustained drug release in different therapeutic applications (Mir, Ahmed, & ur Rehman, A., 2017). PLGA can be used for encapsulation of antimicrobial agents, antibiotics, or anti-inflammatory drugs for wound healing purposes (Chereddy, Vandermeulen, & Préat, 2016). PLGA is biodegraded by hydrolysis, which breaks its ester bonds resulting in the formation of both lactic and glycolic acids (Keles, Naylor, Clegg, & Sammon, 2015). Although the acidic degradation of PLGA is considered harmful for applications such as bone tissue engineering (Liu, Slamovich, & Webster, 2006), the slow release of lactate from topically applied PLGA has been shown to accelerate wound repair through enhanced VEGF-induced angiogenesis and migration of endothelial cells as well as increased collagen synthesis (Porporato et al., 2012). Microencapsulation of AMPs in PLGA microspheres increases their efficacy by protecting the AMPs from degrading enzymes and enabling their controlled release (Machado, Abercrombie, You, DeLuca, & Leung, 2013). The encapsulation of LL-37 in PLGA NPs accelerates wound-healing activity through the release of LL-37 and lactate. Importantly, the antimicrobial selectivity of AMPs depends on their net charge, and is affected by environmental pH. At low pH, AMPs exhibit effective killing of Gram-negative bacteria, such as *P. aeruginosa* and *E. coli*, but their sterilizing efficacy against Gram-positive bacteria, such as staphylococci, can decrease (Walkenhorst, Klein, Vo, & Wimley, 2013). Because many skin infections are caused by Gram-positive bacteria (Chiller, Selkin, & Murakawa, 2001), the antimicrobial efficacy of AMPs-loaded PLGA NPs against skin infections should be carefully evaluated. In fact, use of AMPs to fight infections caused by resistant Gram-negative organisms could be preferable. PLGA-LL-37 NPs provided high peptide encapsulation (70%) and stability (Chereddy et al., 2014). Chereddy *et al.* demonstrated their efficacy to promote wound closure both *in vitro* and *in vivo*. The PLGA-LL-37 NPs induced migration of keratinocytes and fibroblasts *in vitro* (Fig. 6A), and an increased amount of CD31+ endothelial cells was observed *in vivo* (Fig. 6B). Treatment with LL-37 increases the expression of IL-6, -8, monocyte chemoattractant protein-1 (MCP-1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) as well as their receptors in macrophages. Inncreased expression of IL-6 and VEGF after PLGA-LL-37 NP treatment can help explain their immunomodulatory and pro-angiogenesis effects.

7.3. Wafer formations

Lyophilized wafers are created by freeze-drying of mixed solutions, suspensions, or gels of natural polymers with AMPs or other therapeutic agents. They represent a novel delivery technique for infection control and wound treatment (Pawar, Boateng, Ayensu, & Tetteh, 2014). The wafers can be directly applied onto the wound. Their swelling and flow properties can be modulated by viscosity modifiers, such as Carbopol® resins, pregelatinized maize starch or PEG400, to provide sustained release and maintain steady-state concentrations to cope with either low or high suppurating wounds (Matthews, Stevens, Auffret, Humphrey, & Eccleston, 2005). The most common polymers for wafer preparation to deliver antimicrobial agents include gums like guar, xanthan, karaya, and polymers such as sodium alginate and carboxymethylcellulose. The wafers can provide controlled delivery of AMPs and absorb excessive fluid at the injury site. While they transform from a dehydrated solid porous form into viscous gels, their cargo is released at the infection site through controlled diffusion. New synthetic cationic AMPs (CAPs), NP101 and NP108 hold great potential for the treatment of bacterial, viral, and fungal infections, as well as preventing biofilm formation (O'Driscoll, 2011). Moreover, they show activity against *S. aureus* resistant to methicillin and mupirocin (Mercer et al., 2017). NP108 induced a greater loss of intracellular potassium, as a measure of antibacterial activity (Stephens, 2011), on *E. coli* than on *S. aureus* and *P. aeruginosa*. In wounds, topical controlled-release delivery of CAPs embedded in a freeze-dried wafer produced from guar gum helped to maintain CAP antimicrobial activity (O'Driscoll et al., 2013).

7.3.1. Hydrogel formulations and microgels

Hydrogels are drug delivery carriers of natural or synthetic polymers, and form 3D systems with high water content. A moist environment is one of the important requirements for successful wound healing (Ousey, Cutting, Rogers, & Rippon, 2016). Hydrogels can maintain a moist environment due to the presence of high water content, and can thereby accelerate wound healing (Gupta et al., 2019). The broad applications of hydrogels in the treatment of wound infections and wound healing includes drug delivery of peptides, growth factors, hormones, cytokines, and antimicrobial agents (Hoare & Kohane, 2008). These are essentially useful in commercial wound dressings as they promote the optimal environment required for the healing process owing to the following properties: hydrophilic nature, porous structure, high water content, moist environment, ability to absorb the excessive exudates from the wound, ability to incorporate antimicrobial agents, biocompatibility, transparency, non-adhesiveness with additive effects of cooling, and soothing or anti-inflammatory and/or anti-infective properties (Jones & Vaughan, 2005). Antimicrobial hydrogels represent an attractive tool for the delivery of anti-infectives like antibiotics, AMPs, and cationic peptides (Ng et al., 2014). Hydrogels with antimicrobial activity can be produced by covalent immobilization of AMPs, and they provide good AMP stability without loss of antibacterial activity (Cleophas, Sjollema, Busscher, Kruijtzer, & Liskamp, 2014). For example, anionically charged hydrogels prepared from poly2-hydroxyethyl methacrylate (polyHEMA) can entrap cationic AMPs through electrostatic interaction and extend their activity through controlled release (Brannon-Peppas & Peppas, 1990; Davis & Watson, 1984). Laverty *et al.* have reported the application of polyHEMA-based AMP-loaded hydrogels as protective biomaterials against *S. epidermidis* (Laverty, Gorman, & Gilmore, 2012). Delivered in a hydrogel matrix, the antimicrobial ultrashort peptides, lipopeptide, maximin-4 and C12-Orn-Orn-Trp-Trp-NH20%, demonstrated greater efficacy against *S. epidermidis*

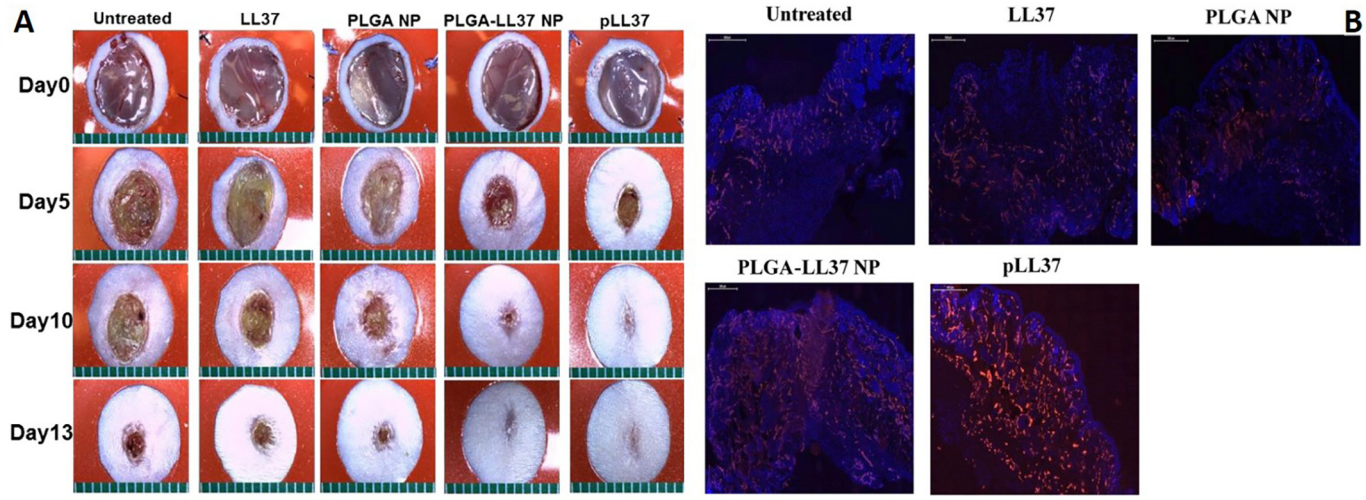

Fig. 6. A. The wound healing by PLGA-LL-37 and LL37 represented in different groups showing wound closure in representative pictures. B. Immunohistochemistry staining of endothelial cells labelled with anti- CD 31 antibody. Key: PLGA, LL-37, Poly(lactic-co-glycolic acid)-LL-37 peptide; NP, nanoparticle; PLGA-NP, poly(lactic-co-glycolic acid) nanoparticles; pLL37, plasmid encoding LL-37; anti-CD 31, antibodies against cluster of differentiation 31. Reprinted with permission from ref. (Chereddy et al., 2014). Copyright 2014, Elsevier B.V.

than vancomycin. Their mechanism of action was based on the prevention of adhesion of *S. epidermidis*, a common Gram-positive pathogen which causes medical device-related infections (Laverty et al., 2012).

Zhou *et al.*(Zhou et al., 2011) developed a photopolymerizable hydrogen based on epsilon-poly-L-lysine (EPL)-grafted broad spectrum methacrylamide (MA) and demonstrated its biocompatibility on human keratinocytes (Zhou et al., 2011). EPL is a positively charged AMP with a hydrophobic segment consisting of -$(CH2)_4$ as a repeating unit that interacts with microorganisms' anionic hydrophobic parts causing membrane damage. The hydrogel produced 3–6 log-fold reduction in bacterial growth and a more than one log-fold reduction in fungal growth. The EPLMA hydrogel caused damage to the microbes through adsorption at the surface of the microbe followed by distortions of their membrane surface and cytoplasm (Fig. 7).

Antimicrobial hydrogels functionalized with AMPs hold great potential in wound infections as dressing materials (Atefyekta et al., 2021). They can be prepared through different techniques including spontaneous self-assembly or through either enzyme-controlled or physicochemical hydrogelation processes to meet the requirements of delivery and tissue engineering applications (Li, Xing, Bai, & Yan, 2019). For example, hydrogels formed by enzymatic crosslinking of synthetic peptides A_9K_2 and I_4K_2 with antimicrobial activity have demonstrated low cytotoxicity and broad-spectrum activity against Gram-positive and -negative bacteria (Bai et al., 2016; Bai, Gong, Wang, & Wang, 2017). Hydrogels are also made from block copolymers composed of repeating blocks of multiple chemically different monomers (Feng, Lu, Wang, Kang, & Mays, 2017). For example polylactic acid (PLA) and its block copolymers are useful in drug delivery and have many biomedical applications like tissue regeneration and imaging due to their good mechanical properties, low immunogenicity, biodegradability, and biocompatibility (Oh, 2011). The block copolymers of PLA/Pluronic are biocompatible and biodegradable and offer a suitable matrix for the controlled release of drugs (Peppas, Bures, Leobandung, & Ichikawa, 2000).

Thermo-responsive *in situ* gels transition from solution to gel form, conform to and seal the injured tissue. They can also be readily applied as injections (Yan et al., 2019). Upon application to the wound as solutions, these *in situ* gels transform into solid gels to then fill the defects and subsequently, upon degradation, deliver their therapeutic payloads. Li *et al.* have synthesized a low-cytotoxic and biodegradable block copolymer and demonstrated the promotion of wound closure by *in situ* injectable gel prepared with this copolymer poly(L-lactic acid)-PluronicL35-poly(L-lactic acid) to encapsulate AP-57 into NPs (Li et al., 2015). AP-57 is a human 57-aa-long, broad-spectrum antimicrobial AMP that is expressed in parts of the gut mucosa as well as in the skin epithelium (Yang et al., 2015). The NPs containing peptide AP-57, prepared by the double emulsion technique, were loaded in thermoreversible gels which upon *in vivo* application transformed from flowing solutions into gels. This transition from solution to gel at the wound site leads to the formation of local sustained drug depot and release of the AMPs. The biomaterial demonstrated low cytotoxicity, near to completely ($96.78\pm3.12\%$) closed the wounds in 14 days, and promoted collagen deposition and re-epithelization in the wound tissue (Fig. 8).

The blending of polymers can help in tuning the toughness and controlling the biodegradability of the polymers. Xie *et al.* designed a partially degradable biomaterial for wound healing applications based on biodegradable PEG maleate citrate (PEGMC) and nondegradable PEG diacrylate (PEGDA) for improving the mechanical properties and degradation rate (Xie et al., 2015). To provide antimicrobial properties, they conjugated the AMPs CHRG01, ALA5, ABU, or TEMP-A to the gels by carbodiimide coupling technique based on the free carboxyl groups in the PEGMC polymer. The antibacterial studies proved that both PEGMC alone or conjugated to any of the peptides, improved infection control compared to untreated wounds. Hydrogels conjugated with ABU or TEMP-A provided higher antibacterial activity as compared to hydrogels with other conjugated peptides. The authors observed significant antibacterial activity of *in situ* forming biodegradable hydrogels against *S. aureus*. The *in vivo* studies in rats demonstrated faster wound repair attributed to increased granulation tissue formation, myofibroblast formation, and collagen deposition compared to the plain polymer. The commercial antimicrobial wound dressing Hydrofera Blue® served as control. As stated earlier, PEGylation is generally used to improve protein delivery and stability (Yan et al., 2019). However, the use of PEG can be limited by its associated activation of the complement system, antibody-mediated hypersensitivity reactions and immunogenecity as a hapten (Knop, Hoogenboom, Fischer, & Schubert, 2010). Dextrins with a biodegradable, non-immunogenic nature can provide an alternative approach to provide stability and controlled release of proteins (Molinos, Carvalho, Silva, & Gama, 2012). The dextrin-conjugated AMP, LLKKK18, incorporation in carbogel demonstrated wound closure and healing potential in rats (Silva et al., 2015). LLKKK18 is a cationic analogue of LL-37. It has greater hydrophobicity and demonstrates enhanced antimicrobial and chemoattractant activities. Silva *et al.* contributed the observed improved wound healing by the LLKKK18 carbogel to reduced oxidative stress (Silva et al., 2015).

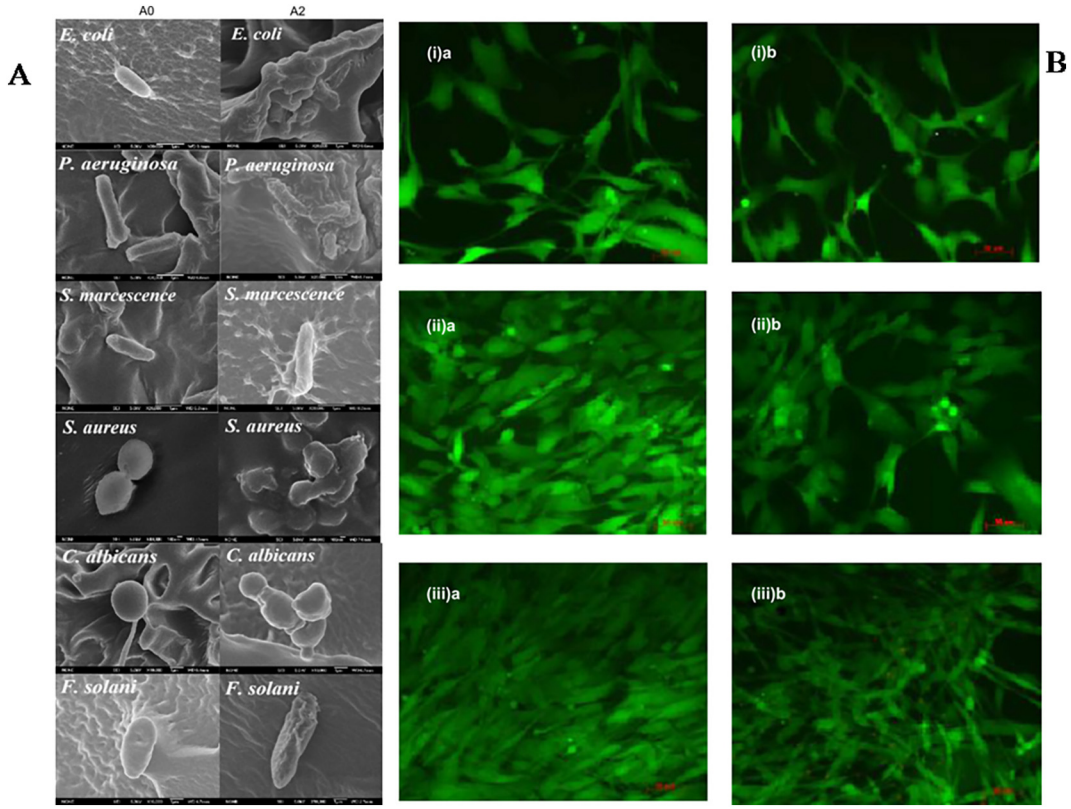

Fig. 7. The effect of antimicrobial hydrogel A. Scanning electron microscope images of microbes on EPLMA hydrogels. A0 control and A2 hydrogel. B. Primary epidermal keratinocytes examination by live/dead cell assays (a) on TCPS control and (b) A2 hydrogel. Key: EPLMA, Epsilon-poly-L-lysine-graft-methacrylamide; TCPS, *tissue culture polystyrene*. Reprinted with permission from ref. (Zhou et al., 2011). Copyright 2011, Elsevier Ltd.

Similar to hydrogels, microgels can be used as carriers for the delivery of AMPs (Borro, Nordström, & Malmsten, 2020). Microgels are colloids sometimes termed as nanogels, microspheres, or even macrogels to describe their particles that vary according to formulation technique, physicochemical properties, and application (Thorne, Vine, & Snowden, 2011). The micro- or nanogels obtained from biopolymers are hydrophilic, cross-linked (either physical cross-linking or chemical crosslinking) and solvent-swollen polymeric networks. Bysell *et al.* have extensively worked on studying the electrostatic interactions between AMPs and microgel systems. The major forces behind peptide binding and deswelling of microgel are electrostatic interactions (Bysell & Malmsten, 2009). Deswelling or compaction of microgels is a known phenomenon that may take place due to changed environmental conditions occurring in highly concentrated microgel suspensions

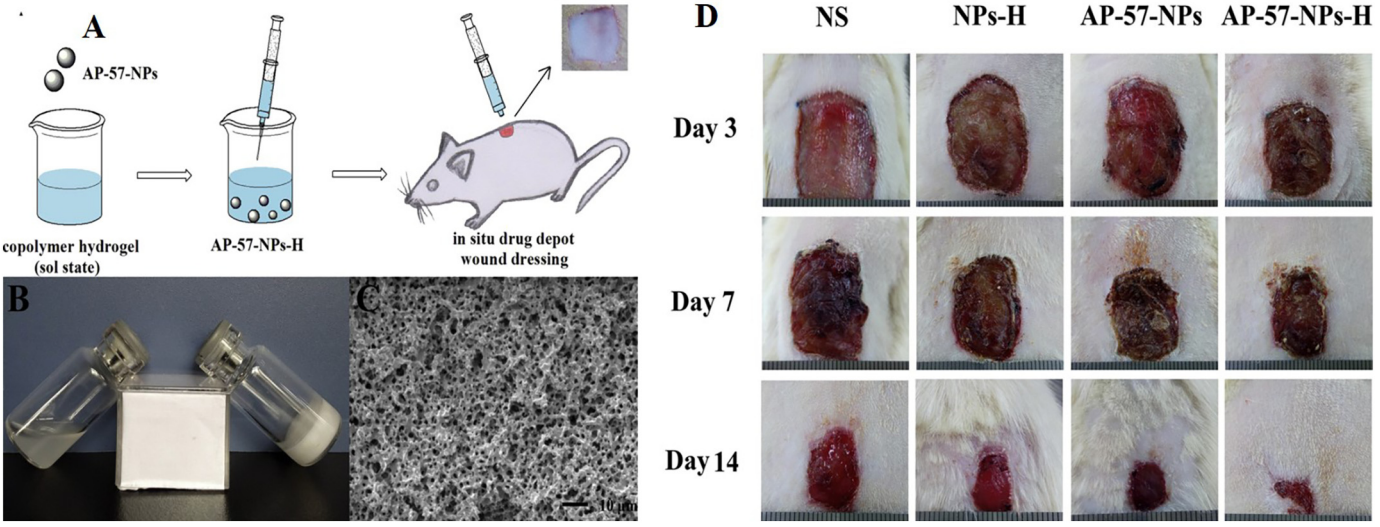

Fig. 8. A. The representation of reversible hydrogel formation encapsulating AP-57NPs B. phase transition from liquid to gel C.SEM image of hydrogel AP-57-NPs-H. D. The wound closure on different days after hydrogel application. Key: AP-57, human antimicrobial peptide; AP-57-NPs, AP-57 loaded nanoparticles; AP-57-NPs-H, AP-57 loaded nanoparticles in hydrogel; NS, normal saline vehicle; NPs-H, nanoparticles in the hydrogel. Reprinted with permission from ref. (Li et al., 2015). Copyright 2015, Elsevier B.V.

(De Aguiar et al., 2017). The antimicrobial effect of human kininogen AMPs, GKH17 and its truncated version KNK10, is reduced at physiological salt concentrations (Nordahl, Rydengård, Mörgelin, & Schmidtchen, 2005; Schmidtchen et al., 2009), and they are readily released from microgels. Hydrophobic end-tagging of the peptides with, for example using tryptophan oligopeptide stretches, resulted in their improved retention in hydrogels (Bysell, Hansson, Schmidtchen, & Malmsten, 2010), and enhanced their antimicrobial potency and stability with lowered cytotoxicity (Schmidtchen et al., 2009). Besides hydrophobicity, other factors that affect AMP interactions with hydrogels are peptide size and structure as well as their charge density (Bysell et al., 2010).

7.3.2. Electrospun fibers (nanofibers)

Nanofibers are produced by different techniques including phase separation, self-assembly, and electrospinning (Hartgerink, Beniash, & Stupp, 2001). Because of its relative simplicity and low-costs, electrospinning is the most common method for producing fibrous matrices. In electrospinning, a solution or melted polymer with appropriate internal cohesion is ejected from a charged needle or nozzle towards the grounded metal collector under a high voltage electric field. Birefringent fibers are formed as the electric forces between the needle and collector stretch the solution across this space, and as the polymer solvent evaporates during the process (Reneker & Yarin, 2008). The uniformity, alignment and diameter of the fibers (Kai, Liow, & Loh, 2014) can be modified by the choice of substrate and solvent (e.g., polymer concentration, viscosity, surface tension, molecular weight, evaporation rate) and the process environment as well as parameters related to electrospinning (e.g., feed rate, applied electric field, needle type or manifold) (Pelipenko, Kristl, Janković, Baumgartner, & Kocbek, 2013; Yang, Murugan, Wang, & Ramakrishna, 2005). The wide variety of possibilities to control both fiber and resulting material properties provide distinct fiber diameters and porosity which could result in different interactions with eukaryotic and bacterial cells (Abrigo, Kingshott, & McArthur, 2015; Lanno et al., 2020).

Different natural and synthetic polymers or their composite combinations can be used for electrospinning. Frequently used combinations include CH with polyvinyl alcohol (PVA)/ gelatin/ polyethylene oxide and the synthetic polymer PCL with natural gelatin or CH (Preem & Kogermann, 2018). Sheets or mats of electrospun fibers exhibit many benefits for wound-healing applications, including similarity to natural skin surface, high surface area, tunable porosity and adequate gas exchange. They are compatible with different bio-active compounds, peptides and drugs (Boateng, Matthews, Stevens, & Eccleston, 2008) that can be decorated onto the fibers or embedded within the fibers either dispersed or as a core of fiber sheathed by a shell using dual nozzles. The use of electrospinning to prepare polymer matrices for wound dressing applications is well-documented (Agarwal, Wendorff, & Greiner, 2008; Huang, Zhang, Kotaki, & Ramakrishna, 2003). Functionalization of electrospun fibers or fiber mats with AMPs, other antimicrobials or growth factors can at best provide all: wound coverage, infection control and stimulation of wound healing (Felgueiras & Amorim, 2017; T. Heunis, Bshena, Klumperman, & Dicks, 2011). The performance of these fibers in the healing process depends on their porosity, air permeability, and surface wettability. Higher hydrophilicity and porosity are essential for wound healing as cells prefer attaching to hydrophilic surfaces and require space for migration and proliferation (Liu et al., 2010).

Ideally, a nano- or microfiber mat designed for a wound-healing application should allow high drug release rates to maintain the local therapeutic concentrations of AMPs and should be biodegradable apart from being selectively porous and air permeable with sufficient strength and adhesive nature. Such an ideal nanofibrous carrier should feature high surface to volume ratio, high porosity, and ability to be easily functionalized (Huang et al., 2003). The applications of electrospun nanofibers include suture coating, multifunctional dressings, dermal replacements, engineered epidermis, and skin regeneration matrices (Chen et al., 2017). Electrospun nanofibers incorporating LL-37 can retain their antimicrobial activity and provide local controlled release with strong antibacterial activity (Gatti, Smithgall, Paranjape, Rolfes, & Paranjape, 2013). The synthetic HDP dimer A3-APO has shown superior efficacy in the treatment of wound infections caused by *Acinetobacter baumannii* and *E. coli* over conventional antibiotics (Ostorhazi et al., 2010). In one study, the A3-APO peptide was loaded into PVA nanofibers which were then used as solid patches for wound healing in mice infected with a high dose of MDR *A. baumannii*. The wound healing improved in terms of wound size, bacterial load, and wound appearance with regenerated-epithelium and granulocytic infiltration when treated with APO monomer patch compared to PVA nanofibers (Sebe et al., 2016). Heunis *et al*. tested nanofiber wound dressings composed of polyethylene oxide and poly (D, L-lactide) (50:50) loaded with commercially available nisin (Nisaplin) (Heunis, Smith, & Dicks, 2013). Nisin is a bacteriocin formed by the *Lactococcus* and *Streptococcus* species of Gram-positive bacteria. It's a GRAS (generally recognized as safe) peptide used to inhibit the outgrowth of *Clostridium botulinum* spores and toxin formation in cheese. It has wide spectrum activity against drug-resistant bacteria such as methicillin-resistant *S. aureus*, *Streptococcus pneumoniae*, *Enterococci*, *Clostridium difficile and* antibiofilm properties (Shin et al., 2016). Nisin-nanofiber formulations resulted in active diffusion of nisin for up to 4 days and were active against MRSA infections. The fibers reduced *S. aureus* bioburden from 2.2×10^7 CFU/wound in controls to approximately 4.3×10^2 CFU/wound (Heunis et al., 2013). The nisin-nanofibers also accelerated wound closure as compared to control fibers without nisin and were proven effective against wounds infected with *S. aureus* (Heunis et al., 2013).

To prevent cytotoxicity and preserve the stability of AMPs, they are often immobilized on the surfaces of biomaterials for most potent control of infections (Tan et al., 2014) and to promote wound healing (Pedrosa, Mouro, Nogueira, Vaz, & Gouveia, 2014). Song and coworkers immobilized Cys-KR12, the shortest peptide motif originating from LL-37, on the surface of silk fibroin (SF) electrospun nanofibers using a combination of reactions 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide/sulfo N-hydroxysuccinimide (EDC/NHS) crosslinking and thiol maleimide coupling (Song et al., 2016). In addition to merely immobilizing the AMPs, these reactions align the peptides optimally for best antimicrobial activity (Hilpert et al., 2009). The immobilization of peptides on nanofibers occurs through a covalent linkage between the thiol and maleimide groups (Fig. 9A). It has been shown that the AMPs' attachment on fibers reduces their activity, but does not affect their antimicrobial spectrum (Bagheri, Beyermann, & Dathe, 2009). The nanofibers prepared by Song *et al.* demonstrated bactericidal activity against several pathogenic wound bacteria such as *S. aureus, S. epidermidis, E. coli,* and *P. aeruginosa.* Membranes made from these nanofibers significantly suppressed bacterial growth compared to pure SF, and the inhibitory effect was greater at higher fiber density (Fig. 9B). The Cys KR12-SF fibers supported proliferation of keratinocytes and fibroblasts, and in keratinocytes, they increased the protein expression of involucrin, a marker for differentiation (Fig. 9C). Furthermore, in LPS-stimulated monocytes the fibers reduced the secretion of TNF-α suggesting anti-inflammatory activity. K200-loaded fibers were able to suppress bacterial growth and biofilm formation as compared to the pristine SF fibers which showed attached bacteria in aggregates to create biofilms.

7.4. Lipid carriers

Lipid colloidal systems can enhance the penetration of drugs, peptides, proteins, and other biomolecules across the skin barrier. These carriers are preferred colloidal systems for developing topical formulations due to their well-tolerated biomimetic nature and their ability to reduce excipient toxicity in the formulation (Souto & Muller, 2007).

There is an increasing trend for using lipid systems like liposomes, solid lipid NPs, liquid crystalline carriers, and nanoliquid carriers as effective topical delivery carriers due to their ability to improve the skin transport and solubilization of the pharmaceutical agents (Jain, Patel, Shah, Khatri, & Vora, 2017). AMPs can be encapsulated into liposomes, solid lipid NPs and liquid crystalline carriers to prevent their proteolytic degradation and to provide sustained controlled release with reduced toxicity (Makowski, Silva, & Pais do Amaral, C., Gonçalves, S., & Santos, N. C., 2019).

7.4.1. Solid lipid nanoparticles (SLNs) and lipid vesicles

The loading of peptides into SLNs can have several advantages, such as decreased toxicity (no toxicity of the carrier itself and reduced toxicity of AMPs due to incorporation into the carrier), protection against the physical or chemical degradation of AMPs, improved AMP bioavailability, and a desired sustained-release profile (Almeida & Souto, 2007). Polymyxins are antibiotic polypeptides effective on Gram-negative bacteria, but are associated with high toxicity (Vaara, 2019). Therefore, it is desirable to develop polymyxin controlled release formulations to provide extended release, enhanced bioavailability and reduced toxicity for successful antimicrobial therapy (Dubashynskaya & Skorik, 2020). Servino *et al.* demonstrated that polymyxin-loaded SLNs have small particle size, high encapsulation efficiency (90%), and increased occlusive properties offering protection against air, fluids and harmful contaminants. SLNs were able to provide sufficient and prolonged skin hydration by forming a film which avoided the loss of water. These formulations were effective against resistant strains of *P. aeruginosa* (Severino et al., 2017).

Chronic wounds are characterized by persistent inflammatory activity with high levels of neutrophil-derived elastases (Wolcott, Rhoads, & Dowd, 2008). Cells, including epithelial cells and cells of the immune system, produce proteinase inhibitors that protect them from excessive proteolysis and limit injury and inflammation (Henriksen, 2014). Therapeutic use of proteinase inhibitors, such as serpin A1, inhibit neutrophil elastase activity (Yager et al., 1997) and accelerate wound healing by balancing the inflammatory reaction (Congote, Temmel, Sadvakassova, & Dobocan, 2008). The synergistic combination of AMPs and elastase inhibitors has been shown to increase their bactericidal potential. Fumakia *et al.* demonstrated that administration of LL-37-encapsulated NPs with serpin A1 resulted in sustained activity of the compounds, suppressed inflammation, reduced bacterial infection and promoted wound closure (Fumakia & Ho, 2016). These solid lipid NPs were prepared by modified solvent diffusion double emulsion techniques using glyceryl monostearate with phosphatidylcholine. They have small particle size, high encapsulation efficiency and tend to accumulate into the skin. They provided sustained LL-37-serpin A1 release for days being effective against *E. coli* and *S. aureus* (Fumakia & Ho, 2016).

7.4.2. Liquid crystalline nanoparticles

Lyotropic liquid crystals (LLCs) are formed upon mixing amphiphiles, cosurfactants, and solvent. They are thermodynamically stable at definite conditions of temperature, pressure, and concentration, and can exist in different modifications; collectively termed as LLCs (Goodby, 2005). LLCs are prepared from amphiphilic lipids such as glycerol monooleate (Milak & Zimmer, 2015) and phytantriol (Akbar, Anwar, Ayish, Elliott, & Squires, 2017) which contain internal water channels and are useful for loading polar drugs (in water channels), nonpolar drugs (a lipophilic portion of lipids), and amphiphilic drugs at the polar/nonpolar interface (Lancelot, Sierra, & Serrano, 2014). These LLCs exist in three main 3D structures termed lamellar, hexagonal, and cubic. The preferred forms are cubic and hexagonal that protect hydrophilic proteins and peptides against proteolytic degradation and provide sustained release (Rizwan, Boyd, Rades, & Hook, 2010). The

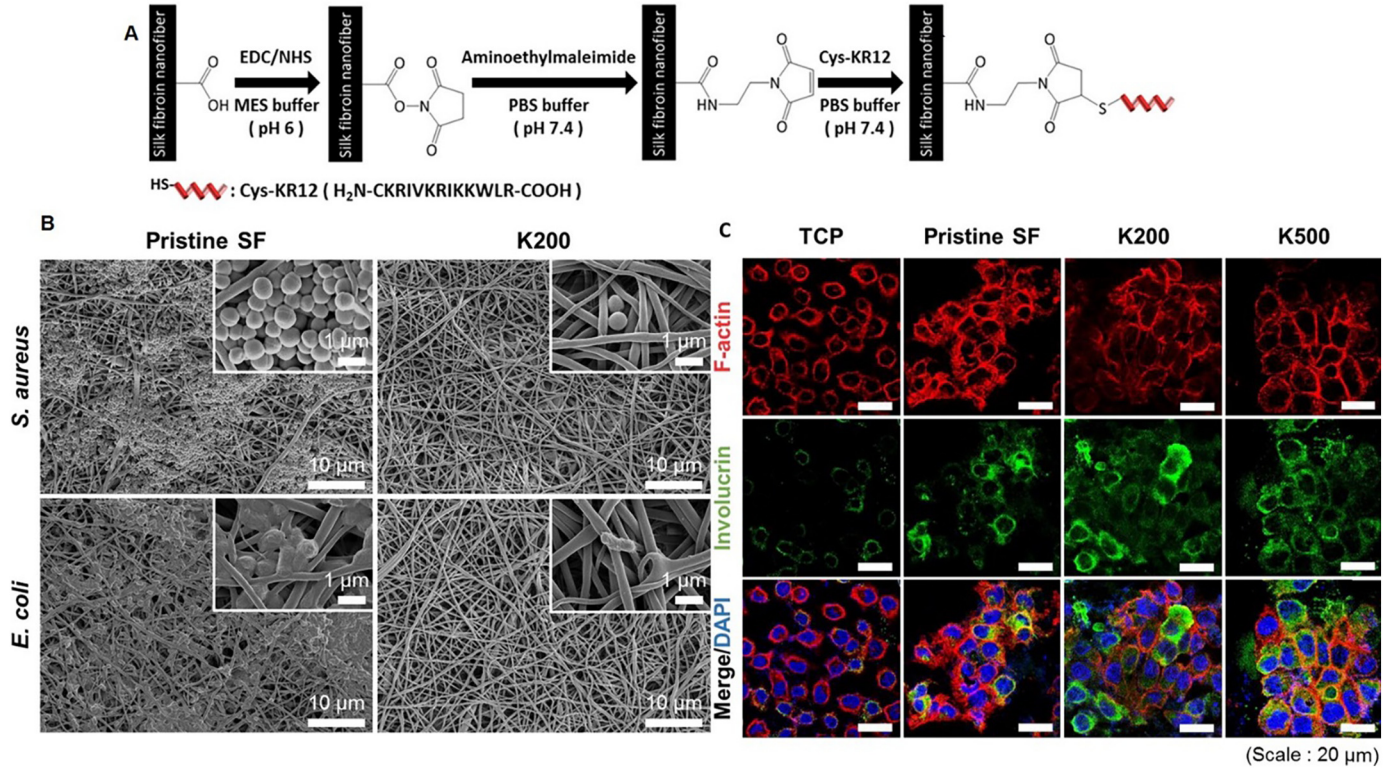

Fig. 9. A. The immobilization of Cys-KR12 peptide on silk nanomatrix B. FE-SEM images of S. *aureus* and *E. coli* cultured on silk fibers and K200 C. Confocal immunofluorescence images of human keratinocytes cultured on TCP, pristine SF, different density fibers K200 and K500 Key: Cys-KR12, antimicrobial peptide motif; SF, silk fibroin; EDC, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide; NHS, N-hydroxy succinimide; pristine SF, pure silk fibroin; TCP, Tissue culture plate; K200, K500, immobilization density; DAPI-4′,6, diamidino-2-phenylindole; FE-SEM, field-emission scanning electronic microscopy. Reprinted with permission from ref. (Song et al., 2016). Copyright 2016, Acta Materialia Elsevier Ltd.

bulk gels can be disintegrated into liquid crystal nanoparticles (LCNPs) using suitable stabilizers. Cubosomes are used to describe the dispersion of the cubic phase, while hexosomes represent the dispersion of the hexagonal phase (Yaghmur & Mu, 2021).

In recent years, the transdermal applications of these carriers have increased, primarily due to their penetration-enhancing effects on the skin's outer layer, *stratum corneum*. They can interact with the lipids of the *stratum corneum* and fluidize the membrane (Esposito et al., 2005; Norlén & Al-Amoudi, 2004). The encapsulation of peptides in cubosomes provides unique opportunities by providing stability through encapsulation. Loading of the enzyme-sensitive AMP LL-37 onto cubosomes can provide sufficient protection against the elastase enzymes, such as human neutrophil and *P. aeruginosa* elastases, and thus maintaining the LL-37 AMP's bactericidal activity and providing proteolytic stability (Boge et al., 2017). AMPs (specifically AMPs with a high positive net charge and pronounced amphiphilicity) are adsorbed and do not penetrate or diffuse into the cubosomes, thus contributing to the sustained AMPs release (Boge et al., 2017).

Glycerol monooleate (GMO)/water (cubic) and GMO/oleic acid/water (hexagonal) are promising liquid crystalline (LC) carriers that can deliver AMPs with preserved and sustained antimicrobial activity. Boge *et al.*,(Boge et al., 2016) investigated the effect of incorporating AMPs with different hydrophobicities and charges into cubic and hexosomal gels. The cubic gel structure was more affected by the peptide addition compared to hexagonal LC gel. In cubosomes, the peptide charge and hydrophobicity controlled the curvature and phase-transition during the incorporation of AMPs. Hydrophobic AMPs interacted strongly with GMO groups leading to structures with negative curvatures and transition into hexagonal phase. Hydrophilic AMPs, on the other hand, interacted strongly with the tips of the GMO molecules and decreased the negative curvature to prefer more lamellar structures. The incorporation of LL-37 in cubosomes influences its antibacterial effects depending on the concentration and specific transformation of the colloid into different forms, such as sponge, lamellar phase, or micelles (Gontsarik et al., 2016). Boge *et al* also evaluated the different strategies of loading the peptide LL-37 into cubosomes to understand the effect of these loading techniques on the particle size, encapsulation, structure, and stability of cubosomes. The peptide can be loaded in gel and later dispersed in NPs or it can be added to preformed cubosomes, termed as pre-loading and post-loading techniques respectively. Hydrotope loading introduces cargo, for example an AMP, during the spontaneous creation of cubosomes from an ethanol/GMO mixture. Hydrotope pre-loaded cubosomes demonstrated high LL-37 loading capacity, proteolytic protection, and sustained bactericidal activity without skin irritation (Boge et al., 2019).

8. Conclusions and future perspectives

The challenges related to increasing antimicrobial resistance augment and accentuate the need for novel effective antimicrobial therapies. These issues converge and culminate in non-healing chronic wounds characterized by multiresistant infections, polymicrobial biofilms and compromised wound healing. As the populations age, the prevalence of chronic metabolic diseases increases and the incidences of hard-to-heal wounds and wound infections rise. AMPs have clear benefits over traditional antibiotics attributed to their antimicrobial and anti-biofilm activities as well as immense potential as broad-spectrum antibiotics. Moreover, bacteria have reduced tendency to develop resistance against AMPs, and the AMPs harbor immunomodulatory activities. The wound healing-promoting actions of AMPs are attributed to their anti-inflammatory effects and abilities to drive adequate cell proliferation and migration for tissue repair.

The instability and toxicity associated with AMPs during production and clinical use restrict their topical use. The design and introduction of chemical modifications, target-based rationalized approaches and synthesis can enhance the efficacy of future AMPs. Carriers and nanoformulation strategies that protect the AMPs and provide controlled release are needed to expose the AMPs' optimal activities. Improved therapeutic activity of AMP nanoscale delivery through encapsulation, conjugation or functionalization aims at increasing their specificity, biocompatibility, stability, and prolonged release while reducing toxicity. Already currently available smart delivery nanocarriers provide an effective way to stabilize and protect the peptides against enzymatic degradation by encapsulation, sustained release, and prolonged activity.

The effectiveness of novel nanoformulation strategies is still limited by production techniques, use of harsh solvents, physical and chemical degradation of peptides, and cytotoxicity at higher doses. These challenges may be resolved by choosing the appropriate composition and manufacturing strategies for AMPs during the formulation step, which will aid in the avoidance of drawbacks during the scale-up and production phase. Nanomaterials such as polymers, lipids, and inorganic metals facilitate topical delivery through encapsulation and controlled release of AMPs, providing necessary porosity, mechanical strength, and protection from physiological and chemical degradation at the target site. Materials, used to produce such nanocarriers, need to be non-toxic, biocompatible, stable during processing and suitable to be used with AMPs. Although several suitable material candidates are available and in use, some of their disadvantages have increased the need for innovative alternative materials. Depending on the nanocarrier formulation (electrospun fibers *vs* nanoparticles *vs* hydrogels etc) specific physicochemical properties and compatibility with other formulation excipients (e.g. solvents, surfactants) are required. As an additional advantage such materials can also have inherent antimicrobial and wound healing properties of their own. Therefore, there is a need to search for novel materials (polymers, lipids) to be used as nanocarriers. It is of specific interest to understand whether synergistic antimicrobial and immunomodulatory actions of nanocarrier materials and AMPs are present in novel drug delivery systems. The incorporation of metal nanoparticles and antimicrobial polymers into the formulation can provide combined synergistic effects for the protection from degradation, antibacterial activity, and reduced toxicity. The nanocarriers encapsulating AMPs provide a sophisticated way to control the complex wound healing process through antibacterial, anti-inflammatory, cellular migration- and re-epithelization-stimulating, and remodeling actions. The nanoformulation strategies and lessons from nature's antimicrobial defense can be expected to drive and contribute to the development of next-generation antibiotics.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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