Journal of Pharmaceutical Investigation (2023) 53:627–641
https://doi.org/10.1007/s40005-023-00628-9

REVIEW

Antimicrobial strategies for topical biofilm-based wound infections: past, present, and future

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Received: 9 April 2023 / Accepted: 16 July 2023 / Published online: 27 July 2023

Abstract

Background Chronic wound infections are a serious global health concern affecting millions of people. One of the major challenges in treating biofilm-based wound infections is the presence of an extracellular polymeric substance (EPS) that limits the penetration of antimicrobial agents.

Area covered This review focuses on conventional, current, and prospective anti-biofilm therapies for treating topical biofilm-based wound infections. Conventional strategies involving wound debridement, topical antibiotics, pH modulation, and surfactants have limited efficacy owing to the regrowth of bacteria, development of bacterial resistance, and difficulty in pH modulation. Improvements in anti-biofilm strategies involve current treatment modalities such as antimicrobial peptides, photodynamic substances, bacteriophages, quorum sensing inhibitors, nanoparticles, and hybrid hydrogels. Such strategies exhibit potent anti-biofilm effects upon topical application by targeting multiple mechanisms. However, the prospects of microbial resistance are still prevalent. Therefore, prospective strategies, such as Natural Deep Eutectic Solvents (NADES) and Clustered Regularly Interspaced Short Palindromic Repeats interfering system (CRISPRi), are required for effective anti-biofilm therapy of infected wounds.

Expert opinion Strategies that completely eradicate biofilm-forming bacteria at wound sites can promote infection control and subsequent wound healing. Further developments in prospective strategies for topical anti-biofilm therapy for infected wounds are warranted. Our review provides valuable insights into the challenges and advancements in the treatment of biofilm-based wound infections, and highlights the need for ongoing research in this area.

Keywords Antimicrobial · Biofilm · Treatment · Wound infection

Introduction

A breach in the skin barrier function (following accidental injury, burns, metabolic dysfunction, or skin diseases) can lead to the formation of wounds (Kujath and Kujath 2010). Successful wound healing depends on a complex series of coordinated phenomena, including inflammation, proliferation, migration, and extracellular matrix remodeling, all of which occur at different stages (Eming et al. 2014; Hu et al. 2014). The failure of one or more of these underlying mechanisms can lead to persistent non-healing wounds, known as chronic wounds (Demidova-Rice et al. 2012). Venous and arterial diseases, diabetes, and microbial infections are among the primary aggravating conditions leading to the development of chronic wounds (Mustoe 2004).

From a microbiological perspective, the primary function of intact skin is to control the microbial population on its surface and prevent invasion and colonization of the underlying tissue (Bowler et al. 2001). A wound exposes the subcutaneous tissue, creating a warm, moist, and nutrient-rich environment that is favorable for microbial colonization and proliferation (Chang et al. 2020). Several factors can affect the abundance and variety of microorganisms in a wound, including the wound's location and depth, the level

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of tissue perfusion, and antimicrobial efficacy of the host immune response (Bowler et al. 2001). Dermal infections, such as surgical site infections, burns, and non-healing diabetic foot ulcers, affect 9.1–26.1 million people worldwide (Thapa et al. 2020b).

A biofilm is an assemblage of microbial cells that are irreversibly attached to a surface and enclosed in a matrix of primarily polysaccharide material (Cook and Siraj 2017). The different stages of biofilm formation, including surface attachment, microcolony formation, biofilm maturation, dispersal, and detachment, are illustrated in Fig. 1. Biofilms are polymicrobial, and different genotypes are held together by extracellular polymeric substances (EPS) (Flemming et al. 2016). Wound colonization and subsequent infections can involve potentially pathogenic microorganisms, including *Staphylococcus* and *Pseudomonas* (Peters et al. 2012; Ruffin and Brochiero 2019). Biofilms present a significant obstacle to the healing of chronic wounds, as they offer substantial protection from host immunity and are often tolerant to antimicrobial agents, leading to delayed healing (Percival et al. 2015). Furthermore, the production of destructive enzymes and toxins by the biofilms can promote chronic inflammation and inhibit wound healing (Rajpaul 2015). In-vitro studies have reported that keratinocytes treated with conditioned media from *S. aureus* and *P. aeruginosa* inhibit cell proliferation, whereas conditioned media alone from *S. aureus* inhibit cell migration (Jeffery Marano et al. 2015). Moreover, low keratinocyte viability was evident following treatment with bacteria-conditioned media. Importantly, in vivo studies involving *P. aeruginosa* alone (Zhao et al. 2010) or in combination with *S. aureus* (a polymicrobial wound infection model) (Pastar et al. 2013) exhibited a decrease in the wound repair rate of cutaneous wounds in murine, rabbit, or porcine in vivo models (Brandenburg et al. 2015; Chaney et al. 2017; Karna et al. 2016; Mendes et al. 2013). Therefore, there are complex, proximate, and dynamic interactions between the chronic wound microenvironment and biofilm state that sustain each other, posing challenges for the effective treatment of chronically infected wounds. As a result, the continuous development of novel/improved antimicrobial strategies is required. This review focuses on the conventional, current, and prospective antimicrobial strategies for the treatment of topical biofilm-based wound infections.

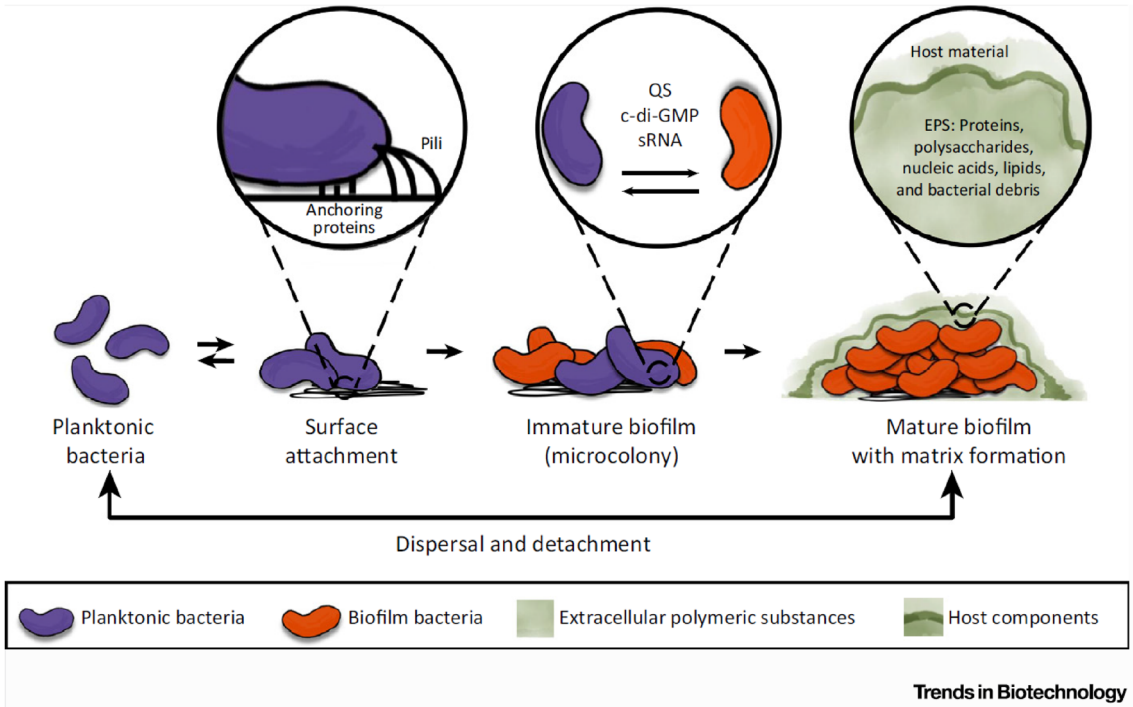

Fig. 1 Stages of biofilm formation. Initial biofilm attachment on biotic surfaces requires specific interactions between planktonic bacteria and the surface via specific anchoring proteins and pili. After successful attachment, planktonic bacteria form microcolonies in which the bacteria transform into a biofilm state via quorum-sensing and sRNA-based systems. Microcolonies gradually mature into a biofilm with a matrix comprising extracellular polymeric substances surrounded by host inflammatory materials and cells. Biofilms eventually form stalk-like structures, which disperse and detach to form new colonies or incorporate into existing biofilms. *c-di-GMP* cyclic-di-GMP; *EPS* extracellular polymeric substances; *QS* quorum-sensing molecules; *sRNA* small non-coding RNA. Reproduced with permission from (Wu et al. 2019)

Antimicrobial strategies for topical biofilm-based wound infections

Biofilm eradication and infection control are crucial strategies to enhance wound healing in chronically infected wounds. However, biofilm infections are often unresponsive to several existing antimicrobial treatments owing to their complex physical and biological properties, multiple microbial genetic and molecular factors, and multi-species interactions (Wu et al. 2019). Therefore, targeting different stages of the biofilm life cycle is necessary to achieve improved therapeutic effects. Figure 2 describes potential treatment options for biofilm-based chronic wound infections at various stages of the biofilm life cycle (Koo et al. 2017). The initial adhesion of microorganisms to the wound site can be targeted by interrupting potential interactions between microorganisms and the wound surface through cell surface-associated adhesins, such as appendages, proteins, and EPS. The early stages of biofilm formation can be inhibited by targeting EPS production and cell division. The developed biofilms can be disrupted by physical removal, EPS matrix degradation, a pathogenic microenvironment (hypoxia or low pH), social interaction targeting, or elimination of dormant cells. Finally, EPS matrix remodeling or the activation of dispersal mechanisms can induce biofilm dispersion. An abundance of antimicrobial strategies (conventional, current, and prospective) can be used for biofilm eradication, as summarized in Table 1.

Wound debridement

Debridement involves the removal of necrotic tissue and foreign objects such as biofilms from a wound to promote healing via exposure to viable underlying tissues (Madhok et al. 2013). It helps to reduce bacterial burden within the wound, control inflammation, and promote the formation of granulation tissue (Sieggreen and Maklebust 1997). Wound debridement is one of the first key steps in the removal of biofilms. In chronic wounds, the body's natural response to necrotic tissue is slow and time consuming. Therefore, a variety of wound debridement techniques can be employed in clinical practice, including enzymatic, conservative sharp and surgical, biodebridement, and mechanical techniques (Vowden and Vowden 1999a, b). Enzymatic debridement involves the use of enzymes, such as collagenase-based dressing, and is

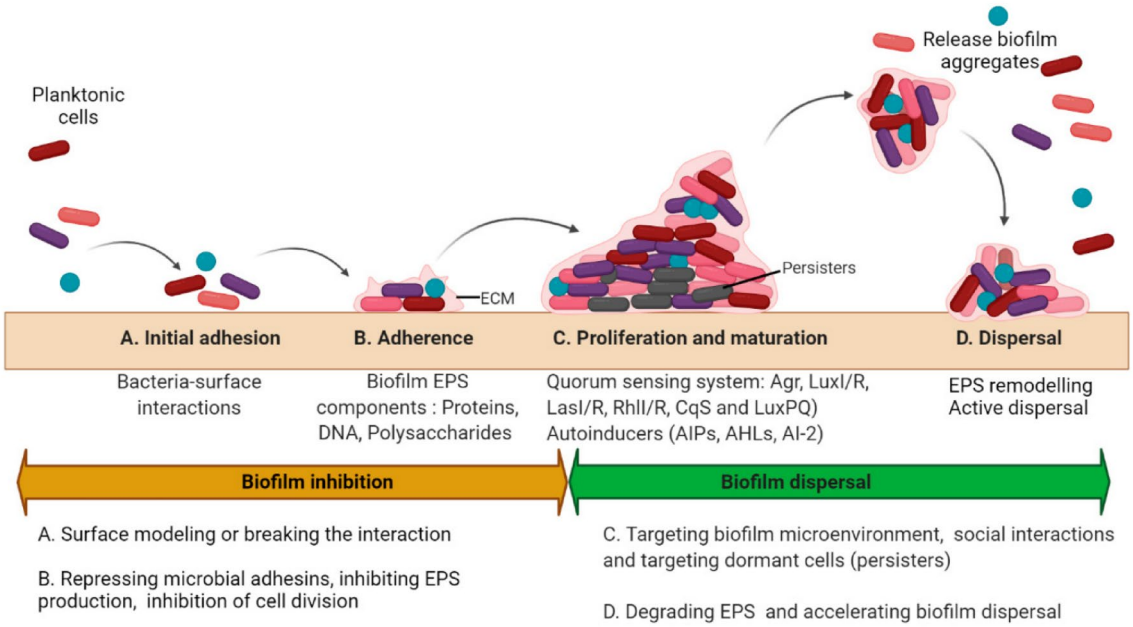

Fig. 2 Biofilm development and anti-biofilm strategies. The microbial biofilm cycle could be classified into 4 phases: Initial attachment, Adhesion, Maturation, and Dispersal. The biofilm inhibitory and dispersal strategies are summarized as per the stages in biofilm development. (A) The initial attachment can be disrupted by interfering with the interactions between the surface and the microorganism either by surface remodeling or physical removal of the biofilms; (B) Adhesion can be inhibited by targeting biofilm EPS and cellular division; (C) Disruption of biofilms in proliferating and maturing phase may be accomplished either by physical removal or by damaging the EPS matrix primarily by affecting the formation of pathogenic microenvironments (such as hypoxia or low pH), and quorum sensing along with the eradication of persister cells. (D) Biofilm dispersal could be achieved by remodeling the EPS matrix or accelerating the dispersal mechanisms. Reproduced with permission from (Koo et al. 2017)

Table 1 Conventional, current, and prospective anti-biofilm agents and strategies

Anti-biofilm agent/strategy	Biofilm component target	Biofilm phase target	Development state	Advantages	Disadvantages
Conventional biofilm strategies					
Wound debridement Bekara et al. (2018); Desjardins and Guo (2018); Liu et al. (2017)	Microbial cells, extracellular polymeric substance (EPS)	Mature biofilm	Clinical (NCT01237392) (Phase: Not applicable)	Clears the necrotic cells and biofilms from wounds	Not appropriate for large surfaces
					Not optimal for saving tissue
				Inexpensive and easy-to-administer methods available	Develop uneven wound bed
Antibiotics Bayramov and Neff (2017); Ciofu et al. (2017); Olivares et al. (2020); Sharma et al. (2019)	Microbial cell	All stages	Clinical (NCT00659646) (Phase II)	Well studied and understood	Resistance development
					Cytotoxic effects
				Promising effects of novel combinations	Limited biofilm penetration
				Possible local delivery to enhance therapeutic effects	Might possess limited activity against the dormant population
Modulation of pH Agrawal et al. (2017); Madhusudhan (2016)	Microbial cells, EPS	Early/mature biofilm	Clinical (NCT05132179) (Phase: Not applicable)	Easy to administer onto the wound surface	Difficult to fine-tune the wound bed pH
					pH variations can affect several factors related to the wound
				Minimal risk of resistance development	Wound-specific conditions determine the effects of pH; no universal strategy is possible
Surfactants Das Ghatak et al. (2018); Percival et al. (2017); Yang et al. (2017)	Microbial cells, EPS	All stages	Clinical (NCT01939145) (Phase: IV)	Can be coated in sutures, dressings, and bandages	Generally effective only in conjunction with antibiotics
				Minimal chances of resistance development	
Current anti-biofilm strategies					
Antimicrobial peptides (AMPs) Pletzer et al. (2016); Thapa et al. (2020a)	Microbial cells	All stages	Pre-clinical (Mouse model)	Easy to engineer and optimize the peptides	Charge resulting from amino acids limit transport through EPS
				Ability to disrupt microbial membrane; reduces the chances of resistance development	Difficult to deliver at the infected site
				Activity is broad-spectrum	Degradation by enzymes and via proteolysis
				Targeting is possible	pH and other environmental factors (e.g., light, oxidation, reduction) can affect activity
Photodynamic substances de Melo et al. (2013); Hu et al. (2018); Yin et al. (2013)	Microbial cell	Early/mature biofilm	Pre-clinical (Mouse model)	On-demand and targeted activity	EPS can hinder the activity
					Light source required
				Controlled bioactivation	Limitations in delivery at the infection site and transport into the biofilm

Table 1 (continued)

Anti-biofilm agent/strategy	Biofilm component target	Biofilm phase target	Development state	Advantages	Disadvantages
Bacteriophages Benjamin and Stephen (2015); Brooks and Brooks (2014)	Microbial cell	Early/mature biofilm	Clinical (NCT02116010) (Phase: I, II)	Specificity and can penetrate biofilm EPS due to small size	Hindered delivery due to strain replacement
					Complications in delivery to the infected site and transport into the biofilm
Quorum sensing (QS) inhibitors Gilles and Tom (2015); Rémy et al. (2018)	Microbial cell	All stages	Pre-clinical (Mouse model)	Manipulate natural pathways to minimize resistance development	Complexity of biofilm signaling network
					Activity dependent on the nutrient source and growth cycle
					Mature biofilm EPS can sequester the signals
				Promote biofilm dispersal and biofilm inhibition	Toxicity to host cells
Nanoparticles organic, inorganic, and hybrid) (Qayyum and Khan (2016); Ramasamy and Lee (2016); Zazo et al. (2016)	Microbial cell, EPS	All stages	Pre-clinical (Mouse model)	Small size permits transport through EPS	Penetration into the EPS can be affected by the charge
					Difficult to deliver at the infected site
				Easy to functionalize	Physiological/biological fluids can affect the properties
				Inherent bioactivity combined with drug-delivery capacity	Can potentially induce cytotoxicity
				Stimuli-responsive or triggered on-demand release is possible	Prolonged retention is required for optimal drug release
Hybrid hydrogels Finnegan and Percival (2015); Palmese et al. (2019); Silva et al. (2019)	Microbial cell, EPS	All stages	Preclinical (Mouse model)	Local application is possible	Accessibility of the released drug or nanoparticles can be limited
				Protection of drugs or nanoparticles from biological fluids and environmental factors until released	
				Controlled/stimuli-responsive drug and nanoparticle delivery can be attained	Potential cytotoxicity
				Potential to be combined with photodynamic therapy	Variability due to chemical compositions
Prospective strategies					

Table 1 (continued)

Anti-biofilm agent/strategy	Biofilm component target	Biofilm phase target	Development state	Advantages	Disadvantages
Natural Deep Eutectic Solvents (NADES) Wikene et al. (2015); Wikene et al. (2017)	Microbial cells	All stages	In vitro	As solvents in antimicrobial photodynamic therapy Enhance the stability of the photosensitizers Potential inherent antimicrobial activity	The NADES network can interfere with the photosensitizer Detailed physicochemical characterization with different drugs is missing
CRISPRi Sharma et al. (2019); Zuberi et al. (2017a, b)	Microbial cells	All stages	In vitro	Interference inhibition of specific genes (e.g., luxS gene expression in *E. coli*) to inhibit biofilm formation Bacteria-specific killing	Difficult to deliver in vivo Complex to translate anti-biofilm effects in vivo

useful during the initial stages of wound management when other techniques are not feasible (Ramundo and Gray 2008). Conservative sharp and surgical debridement, which are currently the gold standard methods, are quick and effective techniques that require a skilled practitioner but are expensive (Bekara et al. 2018). Biodebridement, which involves the use of maggots, has become increasingly popular over the last decade. Larval therapy can be highly selective and rapid but often necessitates the use of other debridement methods following the initial larval application (Gottrup and Jørgensen 2011; Opletalová et al. 2012). Mechanical (wet or dry) debridement is also used; however, it damages healthy granulation tissue, and can be time consuming and painful (McCallon et al. 2015). Various factors, including the type, size, and location of the wound; the nature and volume of the exudate; cost-effectiveness; patient tolerance; and available expertise and equipment, can influence the method of technique. Complete debridement often requires the use of more than one type of debridement. In 2006, the concept of combined debridement was introduced, which involves the use of a combination of methods (e.g., combined sharp and hydrogel debridement, combined ultrasonic and surgical debridement, and combined ultrasonic and enzymatic debridement) to take advantage of complex wounds (Jiang et al. 2009). Although useful for removing biofilms from chronic wounds, debridement techniques can result in uneven and slow healing (caused by tissue removal) of wounds through repetitive processes. Therefore, a suitable combination of debridement techniques and other antimicrobial strategies (e.g., antibiotic or bacteriophage treatments) is required to achieve better therapeutic outcomes.

Antibiotics

Antibiotics are among the most widely used conventional strategies for treating chronically infected wounds. Topical and systemic antibiotics (with broad-spectrum activity) are widely used; however, chronically infected wounds often exhibit poor response to biofilms, which can further lead to the emergence of antibiotic-resistant strains (Hernandez 2006; Lipsky and Hoey 2009). Chronic wound biofilms are antibiotic-tolerant, which is partially attributed to the biofilm construct composed of an EPS matrix that is responsible for poor antibiotic penetration and enzymatic degradation (Høiby et al. 2010; Omar et al. 2017). Furthermore, intrinsic bacterial biofilm factors such as slower growth rates, reduced metabolic factors, and the formation of highly tolerant persister cells and small colony variants contribute to the development of resistance (Stewart 2002). Additionally, environmental factors such as increased oxidative stress, pH variation, and poor oxygenation can decrease the availability, distribution, and resulting efficacy of antibiotics in

chronically infected wounds (Gupta et al. 2016; Percival et al. 2014). Examples of topical antibiotic regimens for cutaneous biofilm infections include mupirocin 2% ointment, metronidazole 0.8% gel, and silver sulfadiazine 1% cream (Ciofu et al. 2017). The therapeutic efficacy of these formulations is often limited by their short residence time, uncontrolled antibiotic delivery, and minimal effects on EPS dispersal. Therefore, suitable modifications of topical antibiotic formulations are required to effectively treat biofilm-based infections. For example, controlled release of antibiotics can enhance the treatment of biofilm-based wound infections. Furthermore, combining EPS-degrading agents (e.g., EPS-degrading enzyme) (Kaplan et al. 2018) with antibiotics in a suitable formulation can enhance the anti-biofilm therapeutic efficacy. A combination of antibiotics can be used to effectively kill biofilm cells by attacking the metabolically active layers (using antibiotics, such as ciprofloxacin, tobramycin, or -lactams) and cells with low metabolic activity (using antibiotics, such as colistin) (Pamp et al. 2008). Antibiotics can also be used to prevent the recurrence of infection following the application of other physical or chemical methods for EPS degradation and removal of biofilms from the wound site. Further modifications of antibiotic formulations and delivery are required to enhance their therapeutic efficacy as anti-biofilm agents.

Modulation of pH

The pH of the wound bed plays a critical role in the healing process of infected wounds, affecting collagen formation, matrix metalloproteinase (MMP) activity, angiogenesis, and immune cell function (Percival et al. 2014). Healthy skin has a slightly acidic pH (4.0–6.0) (Jones et al. 2015) whereas chronic wounds have an alkaline pH (7.15–8.9) that is attributed partly to bacterial proliferation by-products (Jones et al. 2015). This alkaline pH affects the microbial composition (e.g., promotes anaerobic bacterial growth because of low oxygen release) and density (e.g., increasing the density of biofilms by changing bacterial growth rate) (Percival et al. 2012) in infected wounds. Therefore, pH modulation may be a promising strategy for targeting wound biofilms to promote chronic wound healing. Acid treatment is a viable approach, with citric acid (Prabhu et al. 2014), acetic acid (Madhusudhan 2016), and boric acid (Kujath and Hügelschäffer 1987) being studied for their wound pH reduction and anti-biofilm activities. Lowering the wound bed pH can inhibit bacterial proliferation and reduce the toxicity of bacterial end-products, such as ammonia (Leveen et al. 1973). Acetic acid has been effective in both in vitro and clinical settings, particularly against *P. aeruginosa* biofilms (Nagoba et al. 2013); however, it poses a limitation for polymicrobial infections commonly present in chronic wounds.

Surfactants

Surfactants are surface-active agents that lower the surface tension between the liquid and surface, making molecules less likely to stick together. Surfactants play an essential role in wound care by interfering with the ability of microbes to adhere to the wound surface, thus reducing the risk of infection (Percival et al. 2017). Lowering the surface tension between the liquid (used to rinse wounds) and the surface (wound bed) results in liquid infiltration into the surface for the removal of debrided cells and microbes (Percival et al. 2019). One polymeric dressing containing surfactants showed its anti-biofilm effects, which led to the US-FDA approval for the removal of necrotic tissues around wounds (Das Ghatak et al. 2018). Such surfactant-loaded polymeric dressings prevent the biofilm formation by inhibiting aggregation and EPS matrix formation in *P. aeruginosa* and *S. aureus*. Furthermore, a synergistic effect was observed for the combination of antibiotics, resulting from EPS disruption by a surfactant polymer dressing, which converted biofilms into a more planktonic-like phenotype, which could then be cleared with antibiotics (Das Ghatak et al. 2018). Although reports on the use of surfactants as anti-biofilm agents are emerging, a safe and effective dressing or formulation is still required to cover a broad spectrum of biofilm-forming microorganisms.

Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMPs) are short amphiphilic peptides comprising up to 100 amino acids that are present in the first line of defense in organisms (Thapa et al. 2020a). They are part of innate immunity and exert their antibacterial effects through different mechanisms, the most common of which is the formation of pores on the bacterial wall, resulting in leakage and cell death (Lei et al. 2019). Antimicrobial peptides have been studied for their potential roles in the effective treatment of infected wounds. In addition to their antibacterial effects, they also exert immunomodulatory effects, reduce inflammatory components, and induce epithelial cell migration and angiogenesis (Fig. 3) (Thapa et al. 2020a). Although various AMPs have been tested in clinical trials, only a few received market approval owing to their proteolytic instability, pH sensitivity, and high production costs (Dijksteel et al. 2021). Several studies have demonstrated therapeutic potential of different AMPs (innate defense regulator (IDR)-1018 (Mansour et al. 2015), LL37 (Duplantier and van Hoek 2013), DRGN-1 (Chung et al. 2017), and dermaseptin peptide 2 (DMS-PS2) (Song et al. 2020)) for

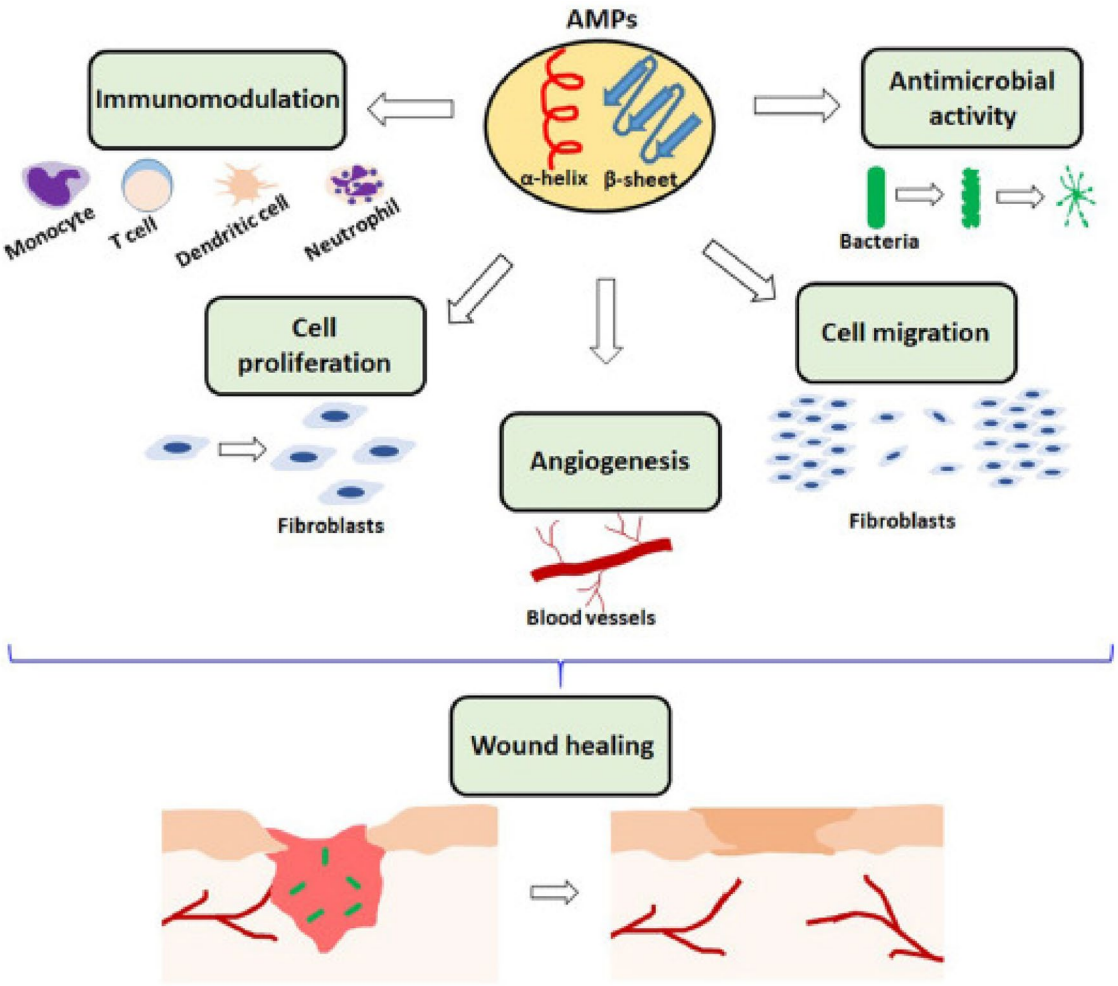

Fig. 3 A schematic representation of the potential biological effects of AMPs on wound healing. Reproduced with permission from (Thapa et al. 2020a)

treating wound biofilms. Nonetheless, their clinical application is hampered due to the lack of an effective manner to prevent their degradation and to control their release, which calls the development of suitable formulation for AMPs to achieve anti-biofilm effects at infected wounds.

Photodynamic substances

Photodynamic substances, also known as photosensitizers (PS), produce cytotoxic reactive oxygen species (ROS) upon harmless visible light irradiation at a particular wavelength, which destroys bacterial biofilms in wounds (de Melo et al. 2013). Antimicrobial photodynamic therapy (aPDT) involves two steps: (1) application of PS to a confined wound area, either locally or systemically, and (2) illumination of the wounded area with a specific wavelength of light to excite the PS and to produce ROS in the presence of ambient molecular oxygen (Hu et al. 2018). Photosensitizers can either be completely sequestered by EPS or partially penetrate the EPS to contact microbial cells (Hu et al. 2018). The excessive ROS leads to oxidative damage to multiple non-specific targets, such as lipids, amino acids, and nucleic acid bases, resulting in cell death induced by damage to microbial proteins, DNA, and membranes (Cieplik et al. 2018). Potential PS for aPDT include acridine orange, tetrapyrrole macromolecules (e.g., porphyrins, chlorins, and synthetic phthalocyanines), non-tetrapyrrole dyes, and natural compounds (e.g., rose bengal, methylene blue, and toluidine blue) (Ghorbani et al. 2018). aPDT has multiple targets by which microbial cells are efficiently killed, and the matrix structure and EPS are weakened owing to the attack of numerous biomolecules (Melo et al. 2021). Nevertheless, the presence of EPS in biofilms and the requirement of a light source to activate PS are considered as downsides of aPDT-based wound treatment. It is typically considered

challenging for PS to penetrate EPS in wound biofilms. A recent study showed that a nanoemulsion containing porphyrin exerted great antimicrobial photodynamic effects with a 6-log reduction of *S. aureus* in an infected ulcer mouse model, demonstrating its anti-biofilm effects that overcame the microbial mechanisms against PS uptake (Buzza et al. 2022). In addition, the electric charge of PS is an important criterion in the successful aPDT. Neutral or anionic PS are effective in eradiation of gram-positive bacteria, whereas they display poor efficacies against gram-negative bacteria due to the electro-repulsive forces between the bacteria's additional asymmetric outer membrane and neutral or anionic PS (George et al. 2009; Sperandio et al. 2013). Such disadvantages could be overcome by cationic PS or conjugation of PS to positively charged entities such as polyethyleneimine (Sperandio et al. 2013). In summary, careful evaluations of the biofilm composition, i.e., whether it is gram-positive or gram-negative, could help to identify suitable PS (cationic, neutral, or anionic), enhancing the therapeutic outcome of aPDT.

Bacteriophages

Bacteriophages are viruses that are specifically designed to infect bacteria via bacterial cell surface receptor recognition and are considered an attractive alternative to combat antimicrobial resistance (O'Flaherty et al. 2009). Bacteriophages can be found abundantly in different environments ranging from soil to the human intestine (Abedon 2009). These viruses have shown effectiveness against a variety of Gram-positive (e.g., *S. aureus* and *Enterococcus faecium*) and Gram-negative bacteria (e.g., *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Vibrio vulnificus*, *Salmonella spp*) (Burrowes et al. 2011). Bacteriophages can be classified as either lytic or lysogenic. Lytic bacteriophages can lyse bacteria, whereas lysogenic bacteriophages are incorporated into bacterial DNA and do not induce bacterial lysis until they are reactivated at a later time, rendering them futile in treating infections (Abedon 2015; Hanlon 2007). Bacteriophages have gained considerable attention for the treatment of biofilms due to their bacteria-lysing abilities (O'Flaherty et al. 2009). Additionally, they can enzymatically degrade the extracellular matrix of the biofilm and spread within the biofilm using endolysins and depolymerases (Tait et al. 2002; Yilmaz et al. 2013). In fact, bacteria-specific phages decrease the areas of bacterial colonization, such as biofilms (Smith and Huggins 1982). The phage titer is proportional to the bactericidal effects and inhibition of the re-emergence of the pathogen (Sabouri Ghannad and Mohammadi 2012). Therefore, phage-mediated bactericidal effects suggest that they can manage wound colonization for safe and effective treatment (Hughes et al. 1998). Several preclinical animal studies have supported the treatment of clinical biofilm infections using bacteriophage therapy, wherein local phage treatment resulted in biofilm reduction (Mendes et al. 2013; Milho et al. 2019; Oliveira et al. 2018; Seth et al. 2013). However, the main concern regarding bacterial lysis by bacteriophages is the release of endotoxins into the wound, which can result in nonspecific and unrestrained activation of innate immunity and inflammatory responses, ultimately delaying wound healing. Other potential disadvantages of bacteriophage therapy include the failure of bacteriophage therapy owing to restrictive specificity, integration of phage DNA into the bacterial genome, and the development of bacterial resistance because of the alteration of bacterial cell surface receptors (Joerger 2003; Sabouri Ghannad and Mohammadi 2012). These disadvantages can be circumvented using a cocktail containing several phages in conjunction with a traditional antibiotic (Comeau et al. 2007). Local delivery of bacteriophages to biofilms in infected wounds is possible using different biopolymers, synthetic polymers, liposomal encapsulation, and inorganic materials (Rotman et al. 2020). Bacteriophage-loaded hydrogels are a potential formulation for effective local delivery to wounds for infection control and healing (Chang et al. 2021; Kim et al. 2021). A recent study reported the development of bacteriophage-loaded nanofibers for the wound treatment associated with *P. aeruginosa* and *S. aureus* (Kielholz et al. 2023). The researchers prepared bacteriophage-loaded nanofiber by electrospinning and showed its antimicrobial effects in the treatment of infected wounds.

Quorum sensing inhibitors

Quorum sensing is a communication system that allows neighboring bacterial cells to receive and send signal molecules (i.e., autoinducers) in a density-dependent manner (Brackman and Coenye 2015). This system plays a pivotal role in biofilm regulation via inter- and intra-species bacterial communication and genetic synchrony (Hentzer et al. 2003b). Therefore, the inhibition of quorum sensing has emerged as a significant innovation in the area of biofilm management (Hentzer et al. 2003a). Such inhibition can be achieved via the degradation of signaling molecules, inhibition of autoinducer synthesis, interference with signal binding, or inhibition of signal transduction cascades, resulting in dysregulated biofilm signaling and subsequent biofilm inhibition or dispersal (Brackman and Coenye 2015; Jiang et al. 2019). Combining these approaches may be necessary to overcome redundancy in bacterial communication and eradicate persisters (Hirakawa and Tomita 2013). Several promising candidates (e.g., furanone, C30, and HT61) have been identified as quorum-sensing inhibitors (Al-Bataineh et al. 2009; Baveja et al. 2004; Pan and

Ren 2013). A quorum inhibitor, FS3, showed good efficacy and synergy when combined with daptomycin (Cirioni et al. 2013). Quorum-sensing inhibitors are effective in managing bacterial biofilms in wounds. However, their efficacy and safety in vivo must be substantiated. Furthermore, increasing evidence suggests the development of a resistant phenotype was initiated by quorum quenching (Gerdt and Blackwell 2014; Scutera et al. 2014), although the mechanism remains unclear. Hence, an appropriate quorum-sensing inhibitor should only be determined after identifying the bacterial species in the wound biofilm. Additionally, other agents, such as antibiotics, should be combined with quorum-sensing inhibitors to enhance the anti-biofilm effectiveness.

Nanoparticles

Emerging advances in nanoparticle-based therapies have provided new and promising opportunities for effectively treating wound infections associated with biofilms. Nanoparticles offer advantages such as cellular penetration, targeted delivery following surface modification, and localized delivery to infected wounds (Andrade et al. 2013). Nanoparticles exhibiting specific physical and chemical properties against biofilm wound infections have also been developed (Kim 2016). Different nanoparticles, such as organic (e.g., liposomes and polymeric nanoparticles), inorganic (e.g., graphene, carbon nanotubes, and mesoporous silica nanoparticles), and metal (e.g., silver and gold nanoparticles), can be used for either the delivery of anti-biofilm agents or for exhibiting intrinsic anti-biofilm activity (Darvishi et al. 2022). A schematic representation of the mechanisms of the different nanoparticle-based approaches for anti-biofilm activity is presented in Fig. 4. Further details on the design, synthesis, and mechanism of anti-biofilm activity can be obtained from recently published review articles (Darvishi et al. 2022; Dizaj et al. 2014). Despite the encouraging prospects for nanoparticle-based anti-biofilm treatment of infected wounds, only a few marketed nanoparticle-based drug delivery systems are available (Duncan and Gaspar 2011). It could be attributed to the complexity in characterization and analysis of nanoparticles. In addition, the limited target selectivity and instability of nanoparticle-based preparations could hinder the clinical successes of nanoparticle-based anti-biofilm therapeutics (Jones and Grainger 2009). Such unmet needs pose an opportunity to develop a new anti-biofilm therapeutic using nanotechnology.

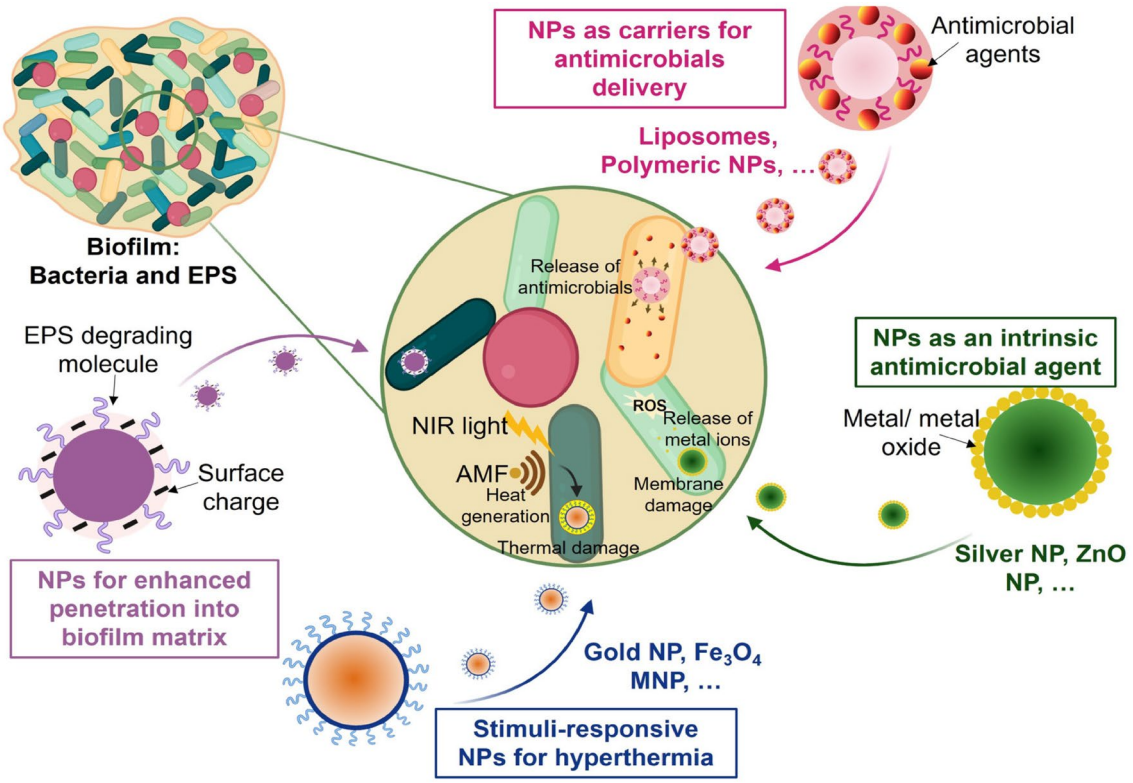

Fig. 4 A schematic representation of the mechanisms of action of various nanoparticle-based treatments for biofilm infections. Reproduced with permission from (Darvishi et al. 2022)

Hybrid hydrogels

Hydrogels are hydrophilic polymer networks that are capable of absorbing, swelling, and retaining large amounts of aqueous fluid (Peppas et al. 2000). They are well-suited for biological applications because of their high water content and permeability, tunable viscoelasticity, and structural similarity to the extracellular matrix (Wichterle and LÍM 1960). Hybrid hydrogels are chemically, morphologically, and functionally distinct building blocks composed of biologically active proteins, peptides, or nano-/microstructures interconnected by chemical or physical means (Palmese et al. 2019). These hybrid hydrogels have potential applications in the successful treatment of biofilms in wound infections. Gelatin hydrogels composed of genipin-cross-linked AgNPs exhibited effective antibacterial and anti-biofilm effects against *S. aureus*, *Bacillus subtilis*, *P. aeruginosa*, and *E. coli* (Katas et al. 2021). Melanin-loaded hybrid hydrogel was developed as wound patches (Cao et al. 2023). It exhibited photothermal antibacterial and antioxidant properties and provided a controlled release of proangiogenic-asiatic acid via liquid transformation of the hydrogel. We also developed a hybrid hydrogel composed of Pluronic F127, liposomes, and antimicrobial peptides exhibiting potent antibacterial and anti-biofilm effects against methicillin-resistant *S. aureus* wound infections (Thapa et al. 2021). The multiple antibacterial and anti-biofilm mechanisms of the hybrid hydrogel components effectively managed wound infections. However, potential toxicity and prevention of resistance development by the infecting bacteria should be taken into consideration.

Natural deep eutectic solvents (NADES)

In addition to water and lipids, natural deep eutectic solvents (NADES) are considered a third class of liquids in organisms and are composed of natural compounds such as amino acids, organic acids, sugars, tertiary amines, and polyols (Grønlien et al. 2020). They are a novel class of eutectics with unique potential as solubilizers of water-insoluble compounds, such as curcumin, for antimicrobial photodynamic therapy against bacteria (e.g., *E. coli*) (Wikene et al. 2015). Although this prospective strategy has potential as an antimicrobial agent in combination with a suitable photosensitizer for photodynamic therapy or as a carrier of an antimicrobial agent, it has some disadvantages, including the potential toxicity of acid-containing NADES (Wikene et al. 2017). Further research using NADES is required to explore their potential in prospective anti-biofilm therapy for infected wounds.

CRISPR interference (CRISPRi)

CRISPR interference (CRISPRi) is a new and prospective approach for quorum sensing inhibition in biofilm-forming bacteria. Several genes (e.g., luxS, fimH, mqsR, csrA, qseB, and motA) are involved in the quorum sensing mechanisms of *E. coli* (Zuberi et al. 2017a). CRISPRi is a gene perturbation technique used for inhibiting genes involved in quorum sensing (Zuberi et al. 2017b). This method reversibly and accurately alters gene expression by hindering transcriptional machinery through the lodging of inactive or dead Cas9 at a specific position (Qi et al. 2013). Although potent in vitro, the CRISPRi method is difficult to translate in vivo to treat wound infections. Further developments and rigorous research are warranted to explore the potential of CRISPRi in the anti-biofilm therapy of infected wounds.

Conclusions and future perspective

The treatment of topical biofilm-based wound infections is a major concern, as millions of people suffer from conditions such as diabetes that can lead to chronic wounds. Various anti-biofilm treatment options, including conventional and current strategies, have been utilized. Conventional strategies, such as wound debridement, antibiotics, pH modulation, and surfactants, are used as anti-biofilm measures. However, their use is limited owing to complications such as bacterial regrowth, limited antibiotic access to bacteria due to EPS, difficulty in pH alteration, and the development of resistant bacteria. Therefore, a combination of different strategies can be useful in synergistically combating the multiple mechanisms of microbial and biofilm wound infections for effective therapy. Furthermore, preclinical studies may hold interspecies differences likely to complicate the precise evaluation of antimicrobial strategies and their therapeutic effects, which requires more rigorous studies in the relevant animal models prior to clinical trials.

Current anti-biofilm treatment options include antimicrobial peptides, photodynamic substances, bacteriophages, quorum-sensing inhibitors, nanoparticles, and hybrid hydrogels. Advancements in treatment modalities, based on intrinsic antimicrobial activity, light-induced ROS generation, controlled release, penetration of anti-biofilm agents into the EPS, and the combination of these anti-biofilm mechanisms within a single formulation, could enhance the potency of these current strategies in treating wound infections. Although promising, the development of resistance is still prevalent, limiting the effective treatment of chronic

wounds. Furthermore, the commercialization of such anti-biofilm treatments is difficult owing to the complexity of treatment modalities, limited stability and modest efficacy, and cost. Rigorous characterization of the modalities, development of stable formulations, and innovative manufacturing process would aid in the clinical translation of new anti-biofilm treatments.

Nonetheless, several prospective strategies have emerged for the eradication of biofilm-forming bacteria at wounded sites. Especially, natural anti-biofilm strategies draw attentions due to their potential resistance against biofilm formation. It includes NADES and CRISPRi technologies. NADES, a natural substance, can act not only as an intrinsic antimicrobial agent but also as a carrier of PS, making itself suitable for combinational anti-biofilm therapy. CRISPRi technology could treat biofilm-forming bacteria selectively. A combination of CRISPRi with appropriate antibiotics could kill the bacteria more effectively at the wounded tissues. Further research on the utilization of NADES and CRISPRi will warrant their potentials for anti-biofilm therapy.

Future efforts for novel treatments for infected wounds require a focus on criteria such as (1) the development of new drugs and therapies, (2) prevention of bacterial resistance, and (3) protection of the natural host microbiome. Discovery of combinations of conventional, current, and prospective therapies will aid in the development of potent anti-biofilm therapies for wound infections. Although evolutionary mechanisms in humans and bacteria may lead to new resistance mechanisms, continuing research will help resolve it.

Acknowledgements This work was supported by the 2023 Yeungnam University Research Grant.

Declarations

Conflict of interest All authors (Raj Kumar Thapa, Jong Oh Kim, and Jeonghwan Kim) declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human and animal subjects performed by any of the authors.

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