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Novel Diagnostic Technologies and Therapeutic Approaches Targeting Chronic Wound Biofilms and Microbiota

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Abstract

Purpose of Review—To provide an up-to-date overview of recent developments in diagnostic methods and therapeutic approaches for chronic wound biofilms and pathogenic microbiota.

Recent Findings—Biofilm infections are one of the major contributors to impaired wound healing in chronic wounds, including diabetic foot ulcers, venous leg ulcers, pressure ulcers, and nonhealing surgical wounds. As an organized microenvironment commonly including multiple microbial species, biofilms develop and persist through methods that allow evasion from host immune response and antimicrobial treatments. Suppression and reduction of biofilm infection have been demonstrated to improve wound healing outcomes. However, chronic wound biofilms are a challenge to treat due to limited methods for accurate, accessible clinical identification and the biofilm's protective properties against therapeutic agents. Here we review recent approaches towards visual markers for less invasive, enhanced biofilm detection in the clinical setting. We outline progress in wound care treatments including investigation of their antibiofilm effects, such as with hydrosurgical and ultrasound debridement, negative pressure wound therapy with instillation, antimicrobial peptides, nanoparticles and nanocarriers, electroceutical dressings, and phage therapy.

Summary—Current evidence for biofilm-targeted treatments has been primarily conducted in preclinical studies, with limited clinical investigation for many therapies. Improved identification, monitoring, and treatment of biofilms require expansion of point-of-care visualization methods and increased evaluation of antibiofilm therapies in robust clinical trials.

Keywords

Biofilm; Microbiome; Host–pathogen interactions; Chronic wounds; Wound infection; Antimicrobials

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Introduction

Biofilms consist of bacteria and fungi organized within a protective layer of extracellular polymeric substance (EPS) matrix comprised of deoxyribonucleic acid (DNA), immunoglobulins, and proteins from both bacteria and host [1]. Recent consensus further defines biofilms as an immunologically protected, genetically diverse microbial community with up to 1,000 times more resistance to antibiotics compared to planktonic bacteria, serving as a source of persistent and recurrent infection [2]. Biofilm infection elicits an inappropriate host inflammatory response, significantly damaging local tissue and skin barrier function. Particularly in chronic wounds, polymicrobial biofilm composition may be influenced by oxidative stress levels in the tissue microenvironment and even by patient genetic variation [3, 4]. Biofilm interaction with the host immune system can lead to persistence of cutaneous inflammatory disease pathogenesis, including in atopic dermatitis [5], acne [6], and hidradenitis suppurativa [7]. Biofilm-driven pathogenesis has been most frequently implicated in chronic wounds including diabetic foot ulcers (DFU), venous leg ulcers (VLU), decubitus or pressure ulcers (PU) and nonhealing surgical wounds [1, 8]. Thus, discussion of diagnostic tools and treatment options herein will focus on wound-based biofilms.

Chronic Wound Microbiome and Host Response

Traditional culture techniques have been found to underestimate the bacterial load and composition of the wound microbiome, especially of anaerobic species. Therefore, microbiome characterization has shifted towards culture-independent techniques such as 16S rRNA sequencing and metagenomic approach [9, 10]. Metagenomic shotgun sequencing of the wound microbiota provides an advantage over 16S sequencing in strain-specific identification, including the ability to discern bacterial isolates with genes providing antibiotic resistance or enterotoxins that correlate to wound healing outcomes [9, 11]. In general, non-healing wounds have lower bacterial diversity than healthy skin [12], with microbial stability correlating to poor clinical outcomes [13••]. Microbiota composition has also been found to differ between ulcer phenotypes based on wound depth, chronicity, and outcomes of healing [10, 14]. In addition to bacteria, 80% of chronic wounds contain fungi, and fungal-bacterial biofilms are associated with poor clinical outcomes [15]. Limited evidence in DFU patients suggests antibiotic treatment does not change overall microbial diversity or abundance [13••]. Antibiotics may even induce a shift in the microbiota to promote virulence factors of pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and further delay wound re-epithelialization. These data suggest the need for careful consideration of antibiotic administration and its potential impact on the wound microbiota.

Bacterial biofilms have been shown to trigger an inflammatory response distinct from that of planktonic bacteria, sustaining chronic wound pathogenesis and bacterial infection (Fig. 1) [16••, 17, 18]. Dysfunction of DFU neutrophils may further contribute to inability of host tissue to respond and clear wound biofilm [19]. Stimulated phagocytic evasion, reactive oxygen species (ROS) production, matrix metalloprotease induction, and resulting collagen degradation damage host tissue and contribute to impaired healing [18, 20, 21].

In addition, biofilm infection downregulates tight junction proteins and compromises the skin barrier to cause increased transepidermal water loss [22]. Most recently, *S. aureus* was identified to have an intracellular niche in DFU epidermal keratinocytes via suppression of innate immune molecule Perforin-2 [23–25]. While it remains to be determined if the intracellular *S. aureus* contributes to biofilm formation, it has been hypothesized to contribute to persistence of DFU infection. Further studies are needed on the complex interactions in biofilms and subsequent impact on the host chronic wound environment to facilitate therapeutic developments targeting biofilm while also stimulating healing.

Diagnostics of Chronic Wound Biofilms

Wound biofilms are a therapeutic challenge in part due to limited accurate clinical identification. While only ~ 6% of acute wounds contain a biofilm, prevalence of biofilm in chronic wounds was found to range from 78 to 100% in patient-focused studies [26, 27]. Electron microscopy is the gold standard for biofilm identification; however, it is a labor- and time-intensive process that is not conducive to clinical point-of-care. Previous clinical indicators for wound biofilms such as presence of slough, exudate, and poor granulation tissue formation have demonstrated low correlation with diagnostic accuracy [28].

Advancement of point-of-care diagnostic tools include the most recent developments in visual markers for enhanced biofilm detection in the clinic (Table 1). One of the methods is application of probes targeting bacteria or biofilm components. For example, an enzyme-based hydrogel involving biomarker alkaline phosphatase changes colors from yellow to purple to indicate detection of *S. aureus* in porcine wound models [29]. Another biofilm detection method is wound blotting and staining. The dyes alcian blue and ruthenium red stain EPS polysaccharides blotted from the biofilm, with alcian blue generally preferred due to quicker staining under a few minutes and increased sensitivity compared to ruthenium red [30]. Beyond hydrogels and dyes, a handheld fluorescence imaging device, MolecuLight *i:X*, uses violet light to stimulate and detect bacteria in wounds. *S. aureus* and *Escherichia coli* emit red fluorescence due to their porphyrin production, while *Pseudomonas aeruginosa* fluoresces cyan due to production of the fluorescent siderophore pyoverdine [31, 32]. Clinical studies have found MolecuLight *i:X* to increase bacterial detection fourfold, with a positive predictive value of up to 92.9% for certain species, significantly improving patient care [32, 33••]. Blotting and MolecuLight *i:X* methods also help localize the distribution of wound infection [30, 34]. With the rapid development of bacterial biofilm identification technologies, visual point-of-care approaches are likely the future of the wound biofilm identification in the clinic.

Pre-clinical Biofilm Wound Infection Models

Murine Wound Biofilm Models

Murine models are the most common animal models used in scientific research due to low cost of acquisition and maintenance [35]. One benefit is their rapid rate of wound healing, allowing shorter experimental timelines. However, wound healing in murine skin primarily uses contractile forces to close wounds, while human skin primarily utilizes re-epithelialization [35]. To establish biofilm in murine models, wounds are often

directly inoculated or infected with pre-formed biofilm [36]. One model utilizes luciferase-expressing bacteria, wherein luminescence released by the breakdown of biofilm EPS and cell lysis signals effects of antimicrobial and biofilm dispersing agents [37]. Biofilm can alternatively be visualized through fluorescent staining or electron microscopy [38]. Diabetic db/db$^{-/-}$ murine models also demonstrate ability to develop wound biofilms under high levels of oxidative stress, using topical antioxidant enzyme inhibitors such as mercaptosuccinic acid and 3-amino-1,2,4-triazole to promote polymicrobial biofilms resembling those of human wounds [3, 39]. While this biofilm model has not been used for pre-clinical assessment of treatments, it has potential for use in future research.

Rabbit Ear Wound Biofilm Model

The rabbit ear is another popular modality to study chronic wound infection as it replicates the ischemia that plays a significant role in developing chronic wounds. An ischemic wound can be created by suturing the arterial blood supply to the rabbit ear and subsequently creating a full-thickness ear wound. The pathogenesis and treatment of infection can then be studied in an ischemic context more closely resembling that of a chronic wound [40]. Like mouse models, rabbits are inexpensive to maintain due to their smaller size and their wounds primarily heal through re-epithelialization [35]. However, anatomical differences still exist from human skin. The dermis is firmly attached to underlying ear cartilage, with an avascular wound base that prevents exact replication of human wound healing. Methods to establish biofilm include monospecies bacterial inoculation into the wound, with impaired wound healing and increased inflammatory cytokines, while topical antibiotics applied 4 days post-wounding to eliminate planktonic bacteria provide improved wound biofilm model in this host [41, 42].

Porcine Wound Biofilm Model

Of all the animal models, the porcine wound healing model is the closest to recapitulating human wound healing. Porcine skin is the most comparable to human skin, with similarities in epidermal thickness, dermal-epidermal thickness ratios, collagen peptides, patterns of hair follicles and blood vessels, and histological location of epidermal keratins 10 and 16, dermal collagen IV, vimentin, and fibronectin [43, 44]. Wound healing also primarily occurs through epithelialization [45]. Drawbacks to experimental use include high costs due to their larger size. However, the large size also allows testing for multiple replicates and therapies within the same experimental animal. In addition to testing multiple novel therapies for biofilm-infected wounds such as antimicrobial nanofiber dressings, electroceutical dressings, and new antibiotics [44], the porcine wound infection model has compared efficacy between different debridement techniques to highlight promising therapeutics that require further investigation in the clinical setting [46].

Novel Therapeutics Against Wound Biofilms

The major challenge in biofilm treatment is that microorganisms are well protected from the host immune response and antimicrobials by several mechanisms including the quorum sensing system, which facilitates bacterial communication to regulate biofilm formation, and the EPS matrix, which impairs treatment penetration and bacterial killing [2]. Successful

suppression of biofilm formation significantly improves treatment efficacy and wound healing outcomes. Below we outline current and developing methods against biofilm including physical disruption, targeting EPS or quorum sensing systems, and nanoparticles for enhanced drug delivery or direct bactericidal effects (Table 2).

Debridement

One of the goals in wound debridement is to reduce bioburden, including necrotic tissue and bacteria, by disrupting the EPS matrix and converting biofilm to planktonic bacteria that is temporarily susceptible to antimicrobial therapy. There are multiple debridement approaches including mechanical, biological (maggot/larval therapy), enzymatic, and ultrasonic methods.

The gold standard to treatment is surgical or conservative sharp wound debridement [2]. However, sharp debridement only temporarily reduces bacterial burden and studies suggest limited removal of microorganisms [22, 47, 48]. Therefore, serial debridement of matured biofilms and use of adjunctive antimicrobial therapy is necessary [2, 49]. Remaining bacteria in deeper layers of the debrided tissue may promote persistent, clinically undetected infection [22]. Hydrosurgical debridement, involving a high-pressure waterjet using up to 15,000 psi, may demonstrate more efficient and precise reduction of bacterial biofilms compared to scalpel use [50]; however, clinical comparison studies have been inconclusive [51]. Additionally, studies have shown significant increased levels of air contamination with bacteria after treatment [50, 52].

Ultrasound debridement involves mechanical low-frequency ultrasonic waves (20–40 kHz) to remove devitalized and necrotic soft tissue while preserving viable tissue [53]. Clinical studies suggest non-contact ultrasonic debridement has limited effect on bacterial burden in wounds compared to contact ultrasound [54]; however, it is hypothesized that effect on wound biofilms is underestimated due to the use of culture-based techniques for identification [55]. Other debridement strategies, including maggot/larvae and enzymatic debridement targeting EPS matrix, have more limited clinical evidence and usage [2]. Lack of visualization of biofilm aggregates is a limitation to complete removal of biofilm during wound debridement, and visual markers discussed above should be utilized to ensure complete removal.

Negative Pressure Wound Therapy

Negative pressure wound therapy (NPWT), also known as vacuum assisted closure (VAC) therapy, utilizes a pump to generate sub-atmospheric pressure in a local area. The negative pressure removes excess exudate, improves blood flow, and reduces bacterial colonization [56]. NPWT has been shown to modulate adhesion factors and quorum sensing systems of *S. aureus*–and *P. aeruginosa*–infected murine wounds, causing decrease in biofilm matrix and more scattered colonies [57, 58]. Some clinical reports support bacterial clearance in diabetic wounds [56], but a systematic review of patient wound studies found no change in bacterial burden based on limited evidence, requiring more clinical studies of NPWT's antimicrobial and anti-biofilm functions [59].

To improve antimicrobial effects, NPWT has been combined with a topical antimicrobial solution delivered to the wound in a regulated, cyclical manner between phases of negative pressure, known as negative pressure wound therapy with instillation (NPWTi). Clinical reports support enhanced effect against bacterial burden compared to just NPWT, particularly in complex wounds such as those with extensive biofilm [58, 60••]. A clinical trial measured decreased nonplanktonic bacteria in chronic ulcers under NPWTi therapy with 0.125% sodium hypochlo-rite solution [61] and other studies demonstrated efficacy with 1% acetic acid, polyhexamethylene biguanide solution, normal saline, and a commercial biofilm-disrupting agent [62–65]. NPWT has been combined with other antimicrobial modalities as well, including silver, with reduced bacterial load in lower extremity wounds of high-velocity trauma patients [66]. Some limitations to NPWT include limited patient mobility due to the patient being attached to the NPWT device, and irritation to peri-wound skin from device adhesion.

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are produced constitutively by many cell types, including resident skin cells, and induced during inflammation or infection, including beta-defensins, cathelicidins (LL-37), and perforin-2 [67]. As widely conserved molecules, AMPs have a broad spectrum of antimicrobial activity and can modulate the host immune system to increase antigen presenting cells, phagocytosis, and suppress inflammatory signaling [67]. They also demonstrate ability to target dormant and intracellular populations, with diminished resistance levels compared to those of antibiotics [24, 68]. However, AMP resistance can eventually develop [69]. Selective antimicrobial activity is also possible through synthetic AMPs containing a binding peptide targeting specific species, which can promote a shift in multispecies biofilm communities to a “healthy” microbiome [70].

Natural and synthetic AMPs specifically act against biofilm by disrupting quorum sensing, inhibiting bacterial adhesion, and promoting biofilm dispersal. One of the first, and most studied, AMPs for anti-biofilm capabilities is LL-37 [71]. LL-37-derived topical gels have demonstrated efficacy against MRSA infection in ex vivo human skin wound models [72]. There are numerous AMPs continuing to be discovered or designed, with databases such as APD (http://aps.unmc.edu/AP/) and DRAMP (http://dramp.cpu-bioinfor.org/) each containing over 2000 entries. Most investigation has been limited to in vitro studies, with a few in vivo animal studies and significantly fewer specifically studying biofilm inhibition.

In the clinical setting, established treatments include cathelicidin AMPs such as colistin (polymyxin E), polymyxin B, and chlorhexidine [73]. Phase III clinical trials of infected DFU found topical AMP pexiganan acetate to have equivalent results as oral antibiotic ofloxacin in microbial elimination rates and wound healing [74]. Currently intravenous Brilacidin, a host defense peptide mimetic, is being tested in a phase II trial for acute bacterial skin infections (NCT02052388). However, overall, translation of AMPs from preclinical to clinical evaluation has been significantly limited. This may be due to interference of the highly proteolytic host microenvironment and reduced peptide stability in vivo, and AMP cytotoxicity at higher concentrations [75]. Bacteria in biofilms can also enact enzymes, signaling, or resistance genes that allow evasion or inactivation of AMP-mediated

bactericidal effects [76, 77]. Peptide stability and antibacterial activity are affected by vehicle of delivery as well, and recent advances in nanostructured antimicrobial peptides (Ns-AMPs) have attempted to improve on such deficiencies [78].

Nanotechnology

One of the largest challenges in biofilm treatment is impaired drug diffusion through the sticky biofilm matrix and dense cellular organization to effectively act on the biofilm structure or pathogenic microbes. Advances in nanotechnology have developed nanoparticles and systems to facilitate diffusion and precision of antibacterial therapies, using particle sizes smaller than biofilm pores and pH sensitivity enabling selective activation in the acidic biofilm microenvironment [79]. These nano-therapies act against biofilm through three main mechanisms: (1) nanoparticles that directly impair bacteria function and biofilm formation, (2) nanocarriers that deliver antimicrobials into biofilm, and (3) physical damage to biofilms.

Nanoparticles made of metal or metal oxide disrupt bacteria function and biofilm formation, including silver, copper, gold, titanium, and zinc [80]. In particular, silver nanoparticles have demonstrated inhibition of quorum sensing virulence factors and biofilm formation, along with antibacterial activity with wound healing in vitro and in vivo [81]. However, there is concern for emerging silver resistance among clinical isolates [82]. A comparative study of metal oxide nanoparticles (ZnO, CuO, and Fe2O3) found zinc oxide to exhibit the most antibacterial effect against multiple bacteria species, and treatment significantly reduced bacterial growth in murine models [83]. However, clinical translation of metal and metal oxide nanoparticles may be limited by cytotoxic effects to host cells such as keratinocytes and fibroblasts [84], and more investigation with in vivo models are needed to address potential nanoparticle toxicity.

The second use of nanoparticles is providing controlled and site-specific delivery of therapeutic agents. Established vehicles such as liposomes and polymeric nanoparticles are reviewed in depth by Forier et al. [85]. Another novel vehicle is vapor nanobubbles, which form around nanoparticles and can locally disturb biofilm integrity to improve antibiotics diffusion [86]. Nanoparticles, lipid-based nano-structures, and nanofiber dressings have demonstrated effective delivery of antibiotics, AMPs, and nitric oxide against biofilms in preclinical models with improved agent stability and action in vivo, although there have been few translated clinical studies thus far [85]. Delivery of photosensitizers into the biofilm for photodynamic therapy (PDT) has effectively impaired *P. aeruginosa*, MRSA, and *S. epidermidis* biofilms in vitro [87, 88]. Clinical studies for patients with infected chronic leg ulcers found that PDT significantly decreased bacteria levels, in correlation with wound healing [89••]. However, PDT may also hold cytotoxic effects against human fibroblasts [88].

Biofilms can also be physically disrupted through thermal or enzymatic damage. Irreversible thermal damage is generated by gold or magnetic nanoparticles (γ-Fe2O3 maghemite and Fe3O4 magnetite), which are activated by near-infrared light or alternate magnetic field [90]. These gold or magnetic nanoparticles also can be conjugated with antibiotics, further

deepening antibiotic penetration into the biofilm [91]. Enzyme-functionalized nanoparticles can degrade biofilm EPS matrix, demonstrated in vitro against *S. aureus* biofilms [92].

Combining multiple antimicrobial and antibiofilm molecules into nano-based therapies allows lower doses of adjuvant drugs due to synergistic effects. However, limitations include costly development and lack of clinical use despite the increasing number of new formulations under laboratorial investigation. Further advances in antibiofilm nanotechnology require more focus on evaluating efficacy and biocompatibility of nanoparticles in vivo and in clinical studies, particularly with understanding potential toxicity and metabolism of the nanoparticles in patients.

Novel Dressings

Dressings are a centerpiece of wound care, promoting an environment favorable to healing by maintaining moisture, thermally insulating, allowing gaseous exchange, and, for some dressing materials, controlling microbial growth [93]. Antimicrobial-impregnated dressings, including alginates and silver, are traditionally useful against superficially infected or high-risk wounds [93]. However, bacterial resistance to silver treatment may represent a challenge [82]. Some newer dressing types with specifically antibiofilm properties include honey-based dressing and electroceutical dressings. Manuka honey–based wound dressings are currently U.S. Food and Drug Administration (FDA) cleared for management of chronic wounds and burns and are commercially available [94]. Its bactericidal activity relates to high methylglyoxal content, but is also likely influenced by other components such as low pH, hydrogen peroxide, and phenolic compounds [95]. Biofilm viability is reduced by inhibiting bacterial adhesion to major extracellular matrix components such elastin, fibronectin, and lamin [96], and synergistic antibiofilm effect has been demonstrated with adjuvant antibiotics in vitro [97]. Chronic wound infections also are effectively treated with manuka honey when introduced on scaffolds such as hydrogels and microneedles [98, 99]. However, the honey's effectiveness varies between bacterial species [95]. A meta-analysis of clinical studies using medicinal honey dressing for DFU found accelerated bacterial clearance rate [100].

The skin naturally contains an electrical gradient, and modulating the host and bacterial electrical forces has become a novel method against biofilm. Presence of an endogenous electric field influences polarization and migration of host cells such as keratinocytes, fibroblasts, and leukocytes [101, 102]. Furthermore, electric signaling plays an important role in bacterial growth, function, and multi-species biofilm formation [103, 104]. Wireless electroceutical dressing (WED), FDA-cleared and available commercially (Procellera®), consists of a matrix embedded with silver and zinc that generates an electric field across the dressing through redox chemical reactions [105]. WED has demonstrated interference of the quorum sensing system, bacterial adherence, and EPS production, inhibiting biofilm-forming bacteria in vitro and disrupting biofilm integrity and biofilm-induced inflammation in vivo [106]. Electronic scaffolds that generate hypochlorous acid also inhibit biofilm formation with minimal damage to surrounding host tissue. Initial clinical studies find electroceutical dressing well tolerated with minimal adverse side effects [106], but investigations have been mostly limited to infected acute wounds [107] (NCT01938066,

NCT04079998, NCT00816101) with one clinical trial in progress for biofilm infection in chronic wounds (NCT04794621). In targeting electro-interactions, WED anti-biofilm activity is unlikely to be attenuated by drug resistance from the biofilm-containing bacteria.

Additional topical therapies have emerged with biofilm disruption technology targeting the EPS matrix. Surfactant molecules can disrupt non-covalent forces of microbial aggregates and mature biofilms, playing a role in biofilm detachment and dispersion. A micelle matrix gel (marketed as Plurogel TM, Medline Inc, Northfield, IL) utilizes concentrated surfactant (Poloxamer 188) to impede biofilm development, with potential quorum-sensing interference (reviewed in [108]). Likewise, the proprietary technology of the Xbio product line (marketed as BlastX™, Next Science Inc, Jacksonville, FL), which includes an antimicrobial gel, disrupts metallic bonds and dissolves polymers of the EPS matrix, then uses the product's high osmolarity and surfactant molecule to promote bacterial cell lysis [108]. Some potential limitations include interference of gel ingredients with use of other antibacterial technologies such as silver. Although direct assessment of biofilm or bacterial aggregates in studies has been limited, a case series of patients with non-healing DFUs demonstrated the ability of micelle matrix gel to reduce microbial load and shift the microbial community composition, and Xbio™ based gel has enhanced healing in multiple randomized control studies of patients with chronic wounds [109–111].

Phage Therapy

Phage therapy uses a virus that can degrade the biofilm by inducing protease synthesis and targeted bacterial cell lysis [112]. Preclinical studies demonstrate efficacy in destroying biofilms and lysing bacteria of specific strains, sometimes using a cocktail of different phages to act on a broad range of bacterial isolates, while sparing the normal skin microbiota and maintaining stability on the human skin [113, 114]. Antibiofilm effects of phage therapy can also be enhanced with combination of other therapeutic treatments such as honey and surgical debridement [112, 115]. Despite being a rather recent development, the bacteriophage approach has several clinical studies supporting efficacy against infected chronic wounds. In one randomized clinical trial, topical application of a bacteriophage cocktail (PP1131) reduced bacterial burden in burn wounds infected by *P. aeruginosa* [116••]. There are also clinical reports of treating refractory-DFU with commercial anti-staphylococcal bacteriophage [117], and a prospective study of patients with chronic non-healing wounds treated with custom bacteriophages [118]. Topical application of phages has been primarily used to avoid side effects of systematic use. Studies suggest phages may impact the host immune system, thereby promoting bacterial infection in certain situations [119]. Other risks include development of bacterial resistance or horizontal gene transfer, resulting in the phage promoting virulent bacterial genes or transferring the genes to other pathogenic organisms. Phages also have narrow range of efficacy due to their specificity towards individual bacterial strains, although this limitation is combated through cocktail mixtures acting against multiple bacteria strains.

Conclusion

Interaction between microbial infection and host response shapes the formation and maintenance of a pathogenic biofilm. New therapeutic strategies show promise in preclinical studies against biofilm infection. However, preclinical models have limited involvement of host-mediated responses to biofilm infection, and current animal models are primarily acute infected wounds that are not fully analogous to chronic wounds [120], limiting evaluation of ultimate therapeutic efficacy and risks compared to human studies. For many therapeutics already available in the clinical setting, randomized clinical trials have been limited. Biofilm therapeutics with some clinical evidence include debridement, NPWTi, photodynamic therapy, silver-based dressings, electroceuticals, and phage therapy. Direct investigation of biofilm changes is rare, possibly restricted by a currently limited toolbox for accurate biofilm identification and monitoring methods in clinic. Successful translation of antibiofilm therapies from bench to bedside rests on developing standard experimental models and evaluation methods that will ultimately allow us to effectively test biofilm-targeted therapeutics and treat patients.

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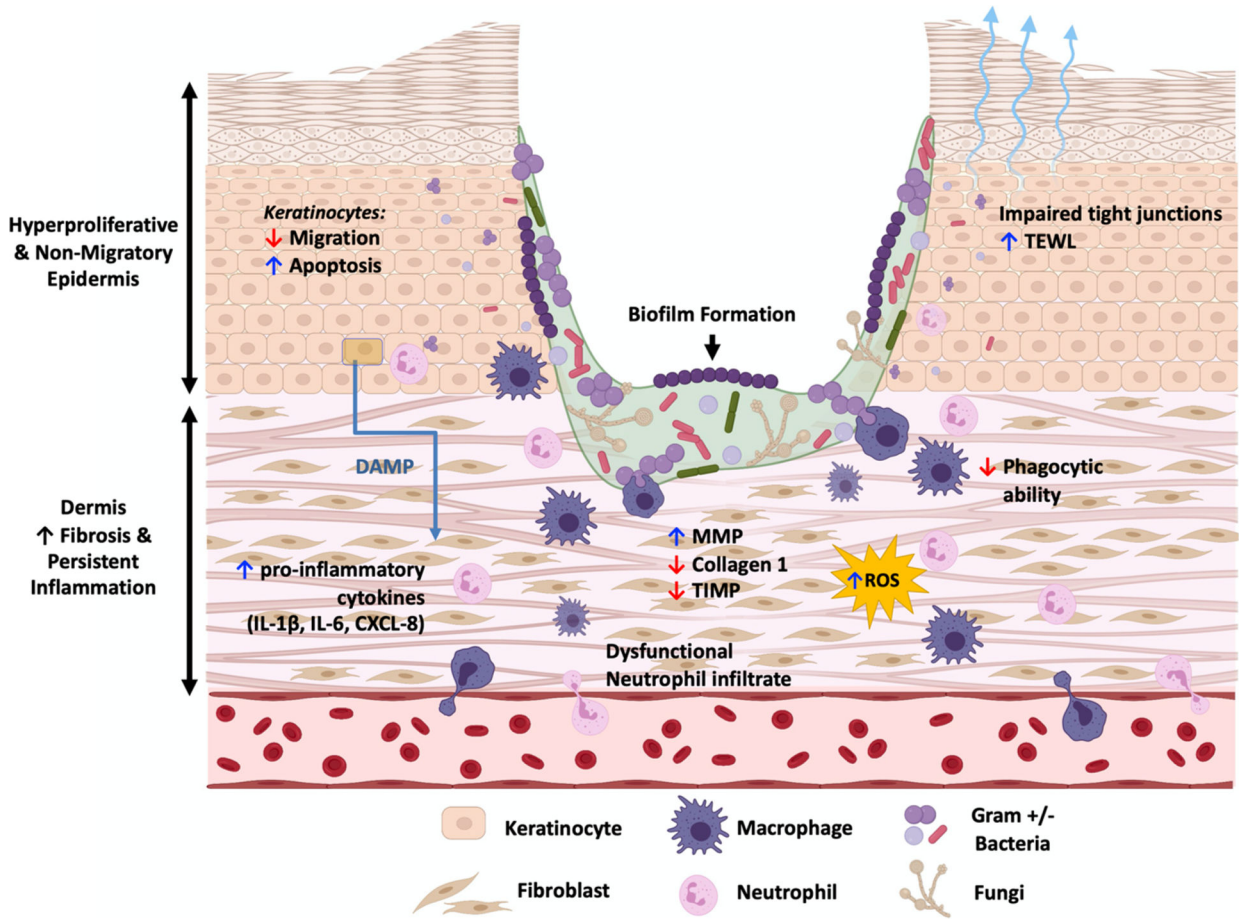

Fig. 1.

Molecular pathology of chronic wound biofilms. Chronic wounds exhibit a hyperproliferative and non-migratory epidermis, unresolved inflammation, and fibrosis. Biofilm presence, which can incorporate a diverse community of bacteria and fungi, promotes impaired keratinocyte migration, dysregulated inflammatory response, and inflammatory cell dysfunction. Additionally, biofilm damages host tissue through increased neutrophilic reactive oxygen species production, imbalance of metalloproteases and inhibitors, and breakdown of keratinocyte tight junctions. These processes further perpetuate chronic wound pathogenesis. (*DAMP*, damage-associated molecular patterns; *MMP*, matrix metalloprotease; *ROS*, reactive oxygen species; *TIMP*, tissue inhibitor of matrix metalloprotease; *TEWL*, transepidermal water loss)

Table 1

Novel biofilm identification methods. Mechanism of diagnostic method, advantages, and disadvantages are summarized for each approach

Diagnostic method	Mechanism	Advantages	Disadvantages	References
Electron microscopy	Tissue biopsy processed and viewed under electron microscope, screen for microbial aggregates and EPS	Gold standard; high level of resolution; visualizes both surface and deep layers of biofilm	Requires invasive wound biopsy; detection limited to sampling location	[27, 28]
Fluorescent probe biomarkers in hydrogel	Fluorescent probe recognizes biofilm marker (e.g., alkaline phosphatase) and induces color change of hydrogel	Noninvasive; prompt detection with color change within 24 h	Probe recognition selectively limited to bacteria type or species; at proof-of-concept stage	[29]
Wound blotting with staining	Blotting the wound transfers biofilm to a nitrocellulose membrane; dyes stain the polysaccharides of biofilm EPS matrix: alcian blue > ruthenium red in sensitivity	Noninvasive; maps biofilm distribution on wound surface; rapid detection with visualization within 2 min	Staining set-up required	[30, 121]
Fluorescent imaging (MolecuLight i:X)	405 nm excitation light emitting diodes shone on wound; fluorescent image captured on device; host tissue appears green, bacteria at > 10^4 CFU/g appear red or cyan	Noncontact, handheld device; rapid detection with image at point of care	Does not distinguish between planktonic and biofilm bacteria	[31–33••, 122]

Table 2

Novel anti-biofilm therapeutic approaches. Mode of action, advantages, and disadvantages of various therapeutics are summarized

Therapeutic method	Mode of action	Advantages	Disadvantages	Ref
Sharp wound debridement	Scalpel for mechanical removal of bacterial aggregates	Improves healing outcomes, increases susceptibility to antibiotics	Temporary reduction; difficulty accessing deeper layers of infection	[49]
Hydrosurgical debridement	High-pressure waterjet for mechanical removal of bacterial aggregates	More efficient compared to sharp surgical debridement	Increased risk of air contamination	[50–52]
Ultrasound debridement	Low-frequency ultrasonic waves applied to wound; non-contact or contact application	Preserves viable granulation tissue, reduced slough and exudate	Variety of devices and settings, limited evidence for an optimal setting	[54, 55]
NPWTi[a]	Vacuum generates sub-atmospheric pressure in wound area; topical antimicrobials delivered between cycles of negative pressure	Improves healing outcomes; enhanced effect compared to NPWT	Limited patient mobility for up to 22 h; skin irritation around wound due to device adhesion	[58, 60••, 61]
AMPs[b]	Molecules with antimicrobial activity that also modulate host immunity; can promote biofilm dispersal through disrupting quorum sensing and adhesion	Large database of potential natural and synthetic AMPs	Reduced peptide stability in vivo; potential cytotoxicity; potential bacterial evasion in biofilm	[74, 76]
Nanotechnology	3 mechanisms: particles that directly impair bacterial function and biofilm, carriers that deliver antimicrobials into biofilm, particles harnessing energy for physical damage	Diffusion through biofilm; can be designed for selective activation; can carry a variety of molecules	Potential cytotoxic effects depending on active molecule	[80, 85, 90]
Honey-based dressing	Bactericidal activity, inhibits bacterial adhesion to extracellular matrix components	Synergistic antibiofilm effect with adjuvant antibiotics	Antimicrobial activity varies between bacterial species	[95, 96, 100]
WED[C]	Electric field generated by redox reaction across dressing, interfering with bacterial electric signaling for biofilm formation	Less risk of acquiring bacterial resistance	Lack of clinical evidence for antibiofilm efficacy	[105, 106]
Micelle matrix gel	Concentrated surfactants disrupt biofilm EPS forces and prevent biofilm formation	Noncytotoxic; less risk of acquiring bacterial resistance	Questionable efficacy against 5. *aureus*	[108, 109]
Xbio™ based gel	Deconstruct EPS matrix's metallic bonds and polymers, lyses bacteria using osmolarity gradient and surfactant	Healing outcomes superior to broad-spectrum antimicrobials; reduced risk of bacterial resistance	Co-application of antibacterial therapies such as silver may interfere with technology	[108, 110, 111]
Phage therapy	Phage virus lyses targets bacterial cells and degrades biofilm matrix	Antimicrobial activity while sparing local microbiota and tissue; customized against specific bacterial strains	Narrow range of efficacy due to specificity; risk of modulating host immune system, resistance, and horizontal transfer of virulence genes	[112, 116••, 117–119]

[a] *NPWTi*, negative pressure wound therapy with instillation

[b] *AMPs*, antimicrobial peptides

[c] *WED*, wireless electroceutical dressing