Biofilm Management in Wound Care

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Learning Objectives: After studying this article, the participant should be able to: 1. Understand the basics of biofilm infection and be able to distinguish between planktonic and biofilm modes of growth. 2. Have a working knowledge of conventional and emerging antibiofilm therapies and their modes of action as they pertain to wound care. 3. Understand the challenges associated with testing and marketing antibiofilm strategies and the context within which these strategies may have effective value.

Summary: The Centers for Disease Control and Prevention estimate for human infectious diseases caused by bacteria with a biofilm phenotype is 65 percent and the National Institutes of Health estimate is closer to 80 percent. Biofilms are hostile microbial aggregates because, within their polymeric matrix cocoons, they are protected from antimicrobial therapy and attack from host defenses. Biofilm-infected wounds, even when closed, show functional deficits such as deficient extracellular matrix and impaired barrier function, which are likely to cause wound recidivism. The management of invasive wound infection often includes systemic antimicrobial therapy in combination with débridement of wounds to a healthy tissue bed as determined by the surgeon who has no way of visualizing the biofilm. The exceedingly high incidence of false-negative cultures for bacteria in a biofilm state leads to missed diagnoses of wound infection. The use of topical and parenteral antimicrobial therapy without wound débridement have had limited impact on decreasing biofilm infection, which remains a major problem in wound care. Current claims to manage wound biofilm infection rest on limited early-stage data. In most cases, such data originate from limited experimental systems that lack host immune defense. In making decisions on the choice of commercial products to manage wound biofilm infection, it is important to critically appreciate the mechanism of action and significance of the relevant experimental system. In this work, the authors critically review different categories of antibiofilm products, with emphasis on their strengths and limitations as evident from the published literature. (*Plast. Reconstr. Surg.* 148: 275e, 2021.)

At any site of infection, microbes are currently known to exist in two distinct phenotypic states: planktonic (free-living) or biofilm (sessile/attached/aggregated). Planktonic microbes can attach to a suitable surface (biotic or abiotic) and develop into polymicrobial biofilm aggregates. Biofilm structures contain aggregated microbes that are encased within a protective polymeric matrix called the extracellular polymeric substance and able to self-adapt to survive in their particular environment.[1,2] The formation of biofilms requires a complex interplay of genetic and environmental (e.g., surface, availability of nutrients) stimuli. It is not clear whether all bacteria have the inherent capability of forming biofilm, the impetus for which is driven by environmental signals that drive genetic changes to initiate biofilm mode of growth. Non–biofilm-forming strains are known to exist and are sometimes used as controls for experimental studies. However, it is theorized that the planktonic mode

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of growth is a laboratory-induced phenomenon in the presence of abundant nutrients and that the biofilm mode of growth is a default mechanism that enables bacterial survival in their natural (nonlaboratory) environment.[3,4] In the biofilm form, microbes have improved tolerance for antibiotics and host immune defenses.[1,5–8] Specifically, they can share plasmids that encode resistance genes, and although some immune cells appear to interact with biofilms (Fig. 1), their function is "frustrated" and incomplete.[9–15] Preclinical studies with *Staphylococcus aureus* biofilms have shown that they produce a cytotoxin called leukocidin that kills neutrophils, rendering them ineffective at clearing the biofilm.[15]

Presently, the wound healing endpoint is based on visual observations. According to the U.S. Food and Drug Administration, a wound covered by skin for at least 2 weeks at two consecutive visits without discharge is clinically determined to be closed.[16] The emergent paradigm of wound biofilm infection has helped uncover a glaring knowledge gap that epithelial covering of wound and lack of discharge may be grossly inadequate to support a decision of wound closure. Even in the presence or history of biofilm infection, the closure rate as determined by wound size may not be significantly impeded, nor will there be any discharge, but restoration of skin barrier function at the site of wound closure is significantly impaired.[17–19] Thus, there is an unrecognized capacity to harm functional wound healing because skin covering the wound cannot perform its critical role as a barrier against infection or regulate evaporative water loss. These studies heighten the need to revisit current clinical standards of a wound closure decision by adding restoration of intact skin barrier functionality as a criterion for healed wounds.[17–19] In addition, biofilm infection severely compromises the extracellular matrix composition (up-regulated collagen-degrading enzymes and inhibited collagen synthesis) of the repaired skin by decreasing the wound-site tensile strength, making it susceptible to wound recurrence.[19]

Several current technologies demonstrate promise in wound diagnostics.[20] Barrier function of the skin can be readily detected at the point of care using transepidermal water loss. Transepidermal water loss detection devices are

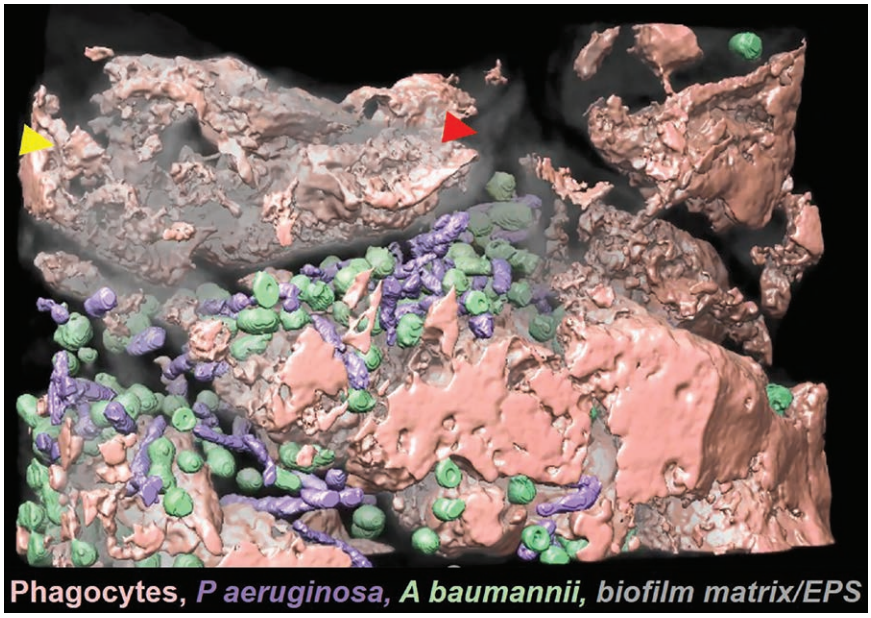

Fig. 1. Three-dimensional imaging of biofilm and host immune cells. Porcine burn wound tissue infected with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* mixed species biofilm were processed and imaged using scanning transmission electron microscopy/focused ion beam/scanning electron microscopic imaging. Shown is a representative three-dimensional image created from individual slices generated by the imager. Phagocytic cells are shown in *pink* interacting with extracellular polymeric substance (*EPS*) (*gray haze*, *red arrowhead*) –coated biofilm aggregates of *P. aeruginosa* (*purple*) and *A. baumannii* (*green*). Some of the phagocytic cells in this image appear to be disintegrating (*yellow arrowhead*).

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U.S. Food and Drug Administration approved for use in dermatologic care and are often used in non–wound-related clinical diagnostics.[21–29] Indeed, observations from ongoing studies in patients with wounds that have been deemed "closed" by a clinician identified deficient barrier function (high transepidermal water loss) in over one-third of all cases. Considered together, these observations lend credence to the notions that (1) restoration of barrier function should be factored in to a functional wound closure decision, and that (2) transepidermal water loss readings could be used as a biomarker of wound recurrence. Currently, National Institutes of Health–sponsored clinical studies are in progress testing the significance of transepidermal water loss in wound care (clinicaltrials.gov NCT02577120).

Biofilm infections are a pernicious factor in human health[30,31] according to the Centers for Disease Control and Infection, the National Institutes of Health, and the U.S. Food and Drug Administration. According to Centers for Disease Control and Infection estimates, 65 percent of all human infectious diseases are caused by biofilm bacteria. The National Institutes of Health estimates that this number is closer to 80 percent.[8] Biofilm formation has been associated with infection of virtually all types of implantable medical devices including but not limited to intravenous catheters in catheter-related bloodstream infections, orthopedic implants, urinary catheters, and craniofacial and dental implants.[5,6,32–48] It is now federally regulated that premarket submissions of medical devices must include antibiofilm strategies.[49] The hunt for antibiofilm solutions in health care has gained momentum.[1,2,50–55] The objective of this work is to discuss antibiofilm strategies used in wound care. [**See Video (online)**, which displays strategies to manage biofilm infection in wound care.]

BIOFILM MANAGEMENT IN WOUND CARE

Strategies to manage biofilm infection in wound care setting may be clustered into three broad categories based on the aspect of biofilm life cycle that is targeted: (1) adhesion inhibitors, (2) biofilm maturation (communication) inhibitors, and (3) promoters of disruption. To achieve these goals, several types of physical, chemical, and biological agents/methods have been tested. None of these has formally risen to the level of standard of care, primarily because of scanty clinical evidence. A few key agents/methods are listed in Tables 1 and 2. Of note, the vast majority of these products have not gone through U.S. Food and Drug Administration clinical trials to specifically secure antibiofilm claim. However, in wound care education sessions at national meetings, products are presented as biofilm-directed management products, with minimal substantiated

Table 1. Traditional Strategies

Strategy	Pros	Cons
Débridement	• Standard technique used by most surgeons • Could be used in combination with other therapies to treat biofilm	• On its own, débridement could push biofilm fragments deeper into wound tissue, promoting chronic wound infection
Silver-based treatments	• Could be used in combination with other therapies that disrupt biofilm to release planktonic bacteria • May be more effective in preventing the initial steps of biofilm formation	• Ineffective against biofilm
Iodine	• Broad-spectrum inhibitory effects of CI against microbial biofilms • Despite the use over many decades, resistance to iodine has been much less of a problem compared to antibiotic therapy	
Physical methods	• Best used on abiotic surfaces such as catheters • Wide variety of options available	• Narrow spectrum of inhibition • May have negative effects against host tissue
Quorum sensing inhibitors	• Wide variety of QS inhibitors or quenchers are available for use in therapeutics	• Narrow spectrum of inhibition in application (specific for the strain being targeted) • Efficacy of these inhibitors have primarily been identified in in vitro studies; the few in vivo studies (amoeba, *Caenorhabditis elegans*, and mouse models) performed have not shown much promise • Possibility of the microbe developing resistance to the inhibitor

CI, cadexomer iodine; QS, quorum sensing.

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Table 2. Emergent Strategies

Strategy	Pros	Cons
Electroceuticals	• Broad-spectrum application to treat a wide variety of pathogenic biofilms either alone or in combination with other treatments such an antibiotics • WED and PED are available in a ready-to-use dressing format that is easy to apply with minimal training • Resistance unlikely	• Apart from WED/PED, other electrochemical approaches may not be practically used • Limited clinical studies
Phage therapy	• Phages are easy to propagate and are highly specific for the bacterial strain targeted • Development of resistance is low and it can be targeted to dormant and persister cells	• The high specificity makes the phage a narrow-spectrum application • Stability and shelf life of phage treatments may be a problem • The concept of using a "virus" to treat bacterial infections is not an easy sell to clinicians
Probiotics	• Broad-spectrum effectivity with low possibility of resistance development • Low toxicity and off-target effects, and inexpensive to produce	• Insufficient clinical evaluations to test the translational value of this intervention as a valid antibiofilm therapy
Antimicrobial peptides	• Broad-spectrum inhibition of Gram-positive and Gram-negative biofilms (IDR-1018, LL-37, DJK-5) • Synthetic AMPs can be modified to provide additional bioactive properties (e.g., RN3)	• AMPs are susceptible to host proteases • AMPs have poor bioavailability • Expensive to synthesize • Insufficient clinical support by means of studies
Dispersal agents	• Could be used in combination therapies where the dispersal agent could disrupt the biofilm and release planktonic cells that can be targeted by antibiotics or other approaches	• Limited in vivo preclinical and clinical studies • Possibility of resistance development against these agents • Potential host toxicity (proteases can cause collateral damage) • Possibility of releasing an abundance of planktonic microbes that could overload the host response system and cause additional pathogenic effects

WED, wireless electroceutical wound dressing; PED, patterned electroceutical wound dressing; AMPs, antimicrobial peptides.

evidence supporting their true application as antibiofilm strategies. Care providers must be mindful of this gap in data and scientific rigor as part of their biofilm education at the present time.

CRITERIA FOR DEFINING BIOFILM INFECTION

Biofilm infection as defined in vivo based on criteria laid out by Parsek and Singh includes the following: (1) aggregate embedded in extrapolymeric substance matrix; (2) adherence to a surface or each other; (3) persistent and localized infection; and (4) resistance to antimicrobial treatments.[56] In addition, a clinically relevant model of biofilm infection must allow for host-microbe interaction under conditions of a competent immune system.[57,58] Scanning electron microscopy is currently a widely acknowledged gold standard to demonstrate polymicrobial aggregates adhered to wound surfaces and embedded in extracellular polymeric substance. Colony-forming unit viability assays are not reliable because these assays do not account for viable but nonculturable persister bacteria, which are metabolically inactive, transient bacterial states with an increased tolerance to stressors, such as antimicrobial therapy and starvation.[59,60] We review the "antibiofilm" strategies below in the context of these criteria to address knowledge gaps the common surgeon working in this area may have. Also interspersed are areas of controversy that are briefly clarified.

CONVENTIONAL STRATEGIES

Débridement

Historically, the management of invasive wound infection included systemic antimicrobial therapy in combination with débridement. Although débridement can be very powerful in debulking hostile biofilm aggregates, lack of visualization of biofilm aggregates during débridement makes it a hit-or-miss type of approach that limits effectiveness. In worse-case scenarios not under the control of the care provider, débridement may inadvertently push the unseen biofilm structures deeper, as demonstrated in a preclinical study where débridement was conducted by a plastic surgeon.[18] A clinical case is presented in Figures 2 and 3. Used alone, débridement may not be sufficient for biofilm removal. However, in combination with other synergistic methods, it could promote chronic wound healing and

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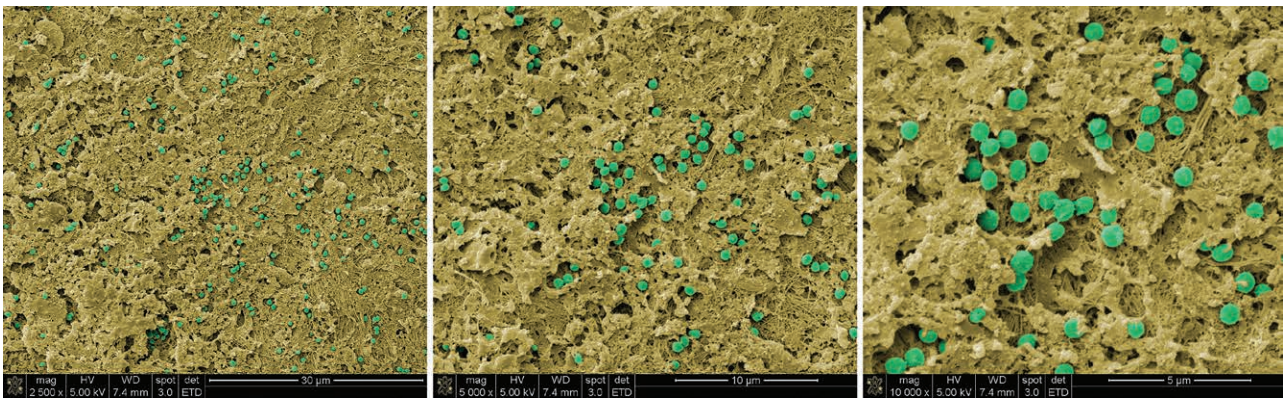

Fig. 2. Aggressive tangential excision is not sufficient to eliminate biofilm infection. An 82-year-old Caucasian man sustained 37 percent total body surface area burns to his left lower extremity and posterior trunk. On presentation to the emergency department, the patient had escharotomy on his left leg and was admitted to the surgical intensive care unit for fluid resuscitation using West Penn formula. Before excision, all burn wounds were dressed with Silvadene (Pfizer, New York, N.Y.). On postburn day 3 he was taken to the operating room for débridement and grafting of his left lower extremity. He had aggressive tangential excision to fascia on the leg and split-thickness skin graft coverage of his lower extremity burns. The postdébridement fascial wound bed was submitted to biopsy and tested positive for biofilm infection by scanning electron microscopy as shown. On postburn day 6, he was taken back to the operating room for excision and grafting of the remainder of his burn wounds on his posterior trunk and thigh. All grafts were treated with Sulfamylon (Mylan, Canonsburg, Pa.) soaks (5% solution). The patient had poor graft take at the site of the wound tissue biopsy with greater than 30 percent graft loss. The patient developed progressive organ failure and died on postburn day 18.

decrease wound recurrence. For example, the use of resorbable antibiotic beads for aggressive antibiotic delivery to débrided pressure ulcers was found to significantly decrease (12.5 percent combination versus 39.4 percent débridement only; $p=0.03$) the recurrence rate of the ulcers.[61] Despite its obvious shortcomings, sharp surgical débridement is still generally considered the gold standard for the management of biofilm because it disrupts the extracellular polymeric substance and converts biofilm bacteria to planktonic bacteria susceptible to antimicrobial therapy for a brief window of time until the biofilm can be reestablished.[62,63]

Maggot Therapy

Maggot therapy, involving the use of maggot excretions/secretions, have been tested using in vitro and ex vivo studies for débridement and shown to be efficient in disrupting biofilms of various bacterial species, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms[64–66]

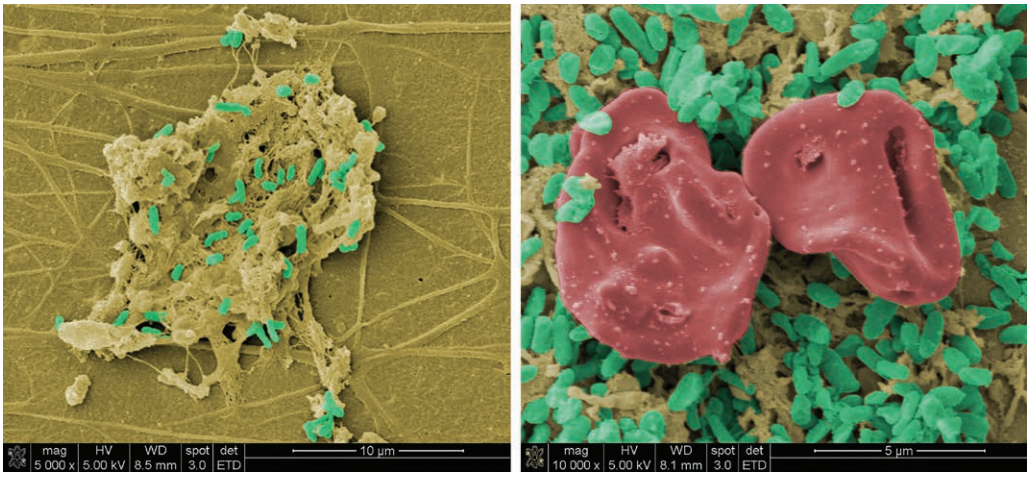

Fig. 3. Biofilm in a central-line catheter taken from an inpatient with burn injury. (*Left*) Island of biofilm cells (*green*) embedded in matrix (*gold*) in lumen of catheter. (*Right*) Collection of bacteria embedded in matrix surrounding red blood cells (*red*) in catheter tip.

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and *Enterobacter cloacae*.[67] However, it was also documented that maggot therapy may be selective in its inhibitory effect. Studies showed that excretions/secretions could enhance or promote biofilm formation of *Proteus mirabilis*.[67] A clinical trial using larval débridement therapy as an antibiofilm therapy was completed in 2018 (clinicaltrials.gov NCT02294175), but no publications are documented as yet.

Ultrasound Therapy

Ultrasound therapy involves the use of low-frequency (20 to 60 Hz) sound waves to clean the wound and directly stimulate immune cells.[68–70] Ultrasound therapy débridement has been investigated as a supportive therapy for chronic wounds, and is hypothesized to both débride the wound and promote healing by increasing cellular activity, promoting synthesis of growth factors, promoting fibrinolysis, and disrupting biofilm.[71–74] Although dispersal of biofilms by ultrasound therapy has been studied in vitro,[75] in vivo studies are limited. One study used colony-forming unit viability assay to assess the effect of ultrasound therapy. There was no significant decrease in bacterial count over the treatment period.[76] At present, it remains speculative whether ultrasound therapy has any impact on biofilm-specific disruption in vivo. Interestingly, low-frequency direct contact ultrasound therapy was found to be effective in dispersing biofilms from metallic implant materials and making them susceptible to disinfectant treatment.[77] The use of ultrasound therapy with microbubbles containing antimicrobial agents is emerging as the next-generation advancement to regular ultrasound therapy and shown to be inhibitory to *S. epidermidis* and *A. baumannii* in in vitro studies.[78,79] Two in vivo studies have been performed using a mouse orthopedic implant model (*Staphylococcus* species) and a rabbit catheter model (*S. epidermidis*) that demonstrated potential synergistic effect against biofilms without exerting toxic effects on the animal host.[80,81] Once developed further, ultrasound therapy (with or without microbubbles) could have promising biofilm-disrupting value for devices and implants.

Physical Methods

Although débridement is a physical method targeting a specific facet of the surgical/operative process, other physical strategies such as nonthermal plasma, photodynamic therapy, and nanotechnology address aspects in the perioperative realm. The mode of action of these methods typically involves preventing adhesion or promoting dispersion; these methods are generally applied to inanimate surfaces or objects.

Nonthermal Plasma

Nonthermal or atmospheric cold plasma involves the generation of photons, electrons, neutrons, and protons when exposed to the constant supply of energy to a gas.[82–84] The antibiofilm effects of atmospheric cold plasma are thought to be attributable to the generation of reactive oxygen and nitrogen species (including organic radicals). Atmospheric cold plasma has been tested in vitro and in a few small-animal studies as an antibiofilm strategy.[82] Clinically, atmospheric cold plasmas are advantageous, primarily because of the ability to control and target the reactive species to cause matrix disruption, quorum sensing inhibition, and induction of dispersal.[82] However, in some cases, plasma–biofilm interactions may result in the development of persisters.

Photodynamic Therapy

Photodynamic therapy involves the use of a nontoxic photoactive dye (e.g., acridine orange, toluidine blue, photofrin[85]) that, when exposed to light of a specific wavelength in the presence of oxygen, becomes activated and produces toxic oxygen species (e.g., free radicals, singlet oxygen). Its use for controlling biofilms has been documented in oral care and has sparked much interest in wound care. Because of the limited penetration capability, photodynamic therapy is possibly most applicable to superficial infections. A few reports have studied the application of photodynamic therapy against bacterial and fungal biofilms both in vitro and in vivo.[86–90] Some unwanted side effects of photodynamic therapy include increased biofilm-forming ability of *S. aureus*.[91] Furthermore, photodynamic therapy can cause allergic reactions and skin photosensitivity at the site of application. The application of photodynamic therapy in the clinical setting for wound care requires significant testing and evaluation.

Nanomaterials

The increased reactivity of nanomaterials (nanometer or submicron scale) and ease of control of their chemical and physical properties[23] has resulted in a surge of interest in use of nanomaterials as a therapeutic option for treatment of biofilm infection. Examples include (1) nanoparticles made of metal or metal oxide that disrupt the cell membrane directly or produce free radicals; (2) controlled and sustained site-specific delivery of drugs using nanoparticles such as liposomes or

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polymeric nanoparticles; (3) physical, irreversible disruption of biofilms using combination therapy such as gold nanoparticles or magnetic nanoparticles [e.g., γ-Fe_2O_3 (maghemite) and Fe_3O_4 (magnetite) nanoparticles] with near-infrared light or alternate magnetic field; and (4) coating surfaces with nanoparticles to prevent adhesion of bacteria and development of biofilms.[92]

Chemical Methods

Silver-Based Management

The use of silver as an antimicrobial agent dates back to ancient Egypt, Greece, and Rome, where silver was used as a metal salt to clean wounds or as threads for sutures. The antimicrobial property of silver manifests when silver is in ionic form. The ionic form of silver has shown effectiveness against bacteria (including methicillin-resistant *S. aureus* and vancomycin-resistant enterococci), viruses, and fungi[93] in planktonic form. On contact with wound exudate, silver ions can be released from dressings into the wound bed and kill the planktonic bacteria. The efficacy of silver-based wound dressings has increased with the advent of silver nanoparticles. Silver nanoparticles are less reactive and less toxic (to human cells) than ionic silver and more applicable to diverse clinical and therapeutic applications.[94]

Several studies have been performed to test the antibiofilm effect of silver. Most of these studies are preliminary because they are in vitro based and test the early stages (e.g., initial adhesion) of biofilm development. Given the known bactericidal nature of the ionic form of silver against planktonic microbes, it is not surprising that these studies show favorable effects on biofilm development. Treating early stages with silver will kill microbes because they are still in the planktonic state and therefore biofilms will not develop. Few in vitro studies have addressed the impact of silver on mature biofilms, including a 2016 study by Bowler and Parsons, where the authors showed the ability of a pH-regulated augmented silver hydrofiber dressing to significantly decrease biofilm.[95] This study is limited by its approach in that it uses a standard colony-forming unit viability assay as a means to claim antibiofilm status. Other studies, including those from our laboratories using a chronic burn biofilm porcine model, have shown that once biofilm is established, silver treatments are of limited benefit.[18,96] Various silver dressings have been tested in porcine models. A silver gelling fiber dressing was used against *P. aeruginosa* wound biofilm in a short-term model (7 days) and demonstrated an apparent decrease in bacterial biomass.[97] The limitations of this study include the absence of standard criteria testing[56] (including the gold standard, scanning electron microscopy) to demonstrate actual biofilm development in the wounds. Furthermore, the short-term study does not address the chronic, persistent nature of a true biofilm and may be preliminary in its findings. Among the limited clinical studies performed with silver-based dressings, no clear biofilm indicators have been tested to validate its antibiofilm capability. Coating medical devices with ionic or metallic silver has not shown much promise, possibly because of inactivation by organic material such as blood.[98] Silver nanoparticle–coated catheters did not allow the biofilm formation by a number of pathogens such as *Escherichia coli*, *Enterococcus* species, *S. aureus*, and coagulase-negative *Staphylococci* when tested in vitro.[99] Clinical studies testing the effects of silver nanoparticle–impregnated coatings on biofilm prevention remain to be reported. Studies have been performed in several small- and large-animal models to show the efficacy of silver nanoparticle–coated stents and catheters in reducing or preventing biofilm infection.[99–102] At high doses, silver nanoparticles could be toxic to human cells.[103] Another serious side effect is increased thrombin formation and platelet activation leading to a thrombosis risk of patients in clinics.[104] Further studies are warranted to address safe applications of silver nanoparticles, particularly in the context of direct contact with human cells and tissues.

Iodine

Iodine is an antiseptic that impacts bacterial cells by multiple mechanisms.[105] The neutral and lipophilic nature of iodine could enhance the penetration of this molecule into biofilms.[105–108] Iodine, like silver, can kill planktonic cells rapidly. However, unlike silver, it is also able to inhibit mature biofilms of *S. aureus*, and *P. aeruginosa* when treated over an extended period. Extended release of iodine beyond the period of strong initial kill may be critical to continue exposing persister cells to antimicrobial molecules, potentially resulting in ultimate death of the persisters and preventing biofilm reformation from these dormant but viable cells. Modern formulations of iodine, particularly in the cadexomer iodine combinations, that sequester iodine without limiting its inhibitory functions, have been shown to have antibiofilm effects and also wound healing capabilities in experimental animal models.[105,108] Rigorous in vivo studies and human clinical trials are warranted.

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Hypochlorous Acid

Hypochlorous acid is known to rapidly eradicate pathogenic bacteria and is less toxic to mammalian cells compared with hydrogen peroxide. Hypochlorous acid has uses as a wound cleansing agent and has been shown to promote wound healing in a rodent model.[109–111] It is the active component of two common wound irrigating agents: Dakin solution and Vashe (Urgo Medical, Fort Worth, Texas). Conflicting evidence (based primarily on in vitro studies) presents an unclear picture about the efficacy of these solutions against biofilms of different bacterial species. One study identified that hypochlorous acid was bactericidal against *Streptococcus* strains but unable to disrupt biofilm.[112] Another study claimed in vivo evidence of the efficacy of hypochlorous acid against biofilm from swab samples and exudates collected from treated venous stasis ulcers. The issue with this study is that it is unclear whether any of these wounds were confirmed as being infected with biofilm-forming bacteria.[113] In this same study, in vitro efficacy against biofilm-forming *Pseudomonas* and *Staphylococcus* strains were demonstrated. It is possible that an antibiofilm effect could be strain specific. Additional studies are required to dissect the true efficacy of this chemical agent against biofilm mode of growth. Hypochlorous acid is thought to be the byproduct of electrical treatment modalities and is briefly discussed in the Emerging Strategies section.

Quorum Sensing Inhibitors

Bacteria communicate to adapt their behavior collectively to their environment by a molecular phenomenon called quorum sensing that involves the synthesis and response to small molecules called autoinducers.[114] Quorum sensing drives the synthesis of virulence factors such as pyocyanin (*P. aeruginosa*), biofilm formation, and other activities.[115] The inhibition of quorum sensing is called quorum quenching. Inhibitors with quorum quenching effect are numerous and range from natural (e.g., certain types of honey[116,117]) to synthetic (e.g., furanones[118–120]) and have been used for direct testing against biofilm-forming bacteria in vitro. Some of these inhibitors have also been considered for treatment of medical devices such as catheters, dressings, and orthopedic and trauma devices,[121] as a means to prevent the development of biofilm.

Natural Products

Manuka Honey

Manuka honey, derived from the manuka tree, has non–hydrogen peroxide–based antimicrobial properties attributed to its high content of methylglyoxal and leptosperin. A few in vitro studies have been performed on biofilm-forming strains using manuka honey alone[122–126] or in combination with antibiotics.[127,128] Reports suggest a synergistic antibiofilm effect of manuka honey together with antibiotics.[129] One report, however, indicated the emergence of persister strains of *P. aeruginosa* in manuka honey–treated samples.[130] Manuka honey–based wound dressings are available on the market and are U.S. Food and Drug Administration cleared for use for the management of chronic wounds and burns.[131] Several have been tested for antibacterial (planktonic) activity in a clinical setting.[132–136] Few in vivo studies to date have specifically addressed the antibiofilm activity of manuka honey.[137,138] Rigorous clinical studies are warranted.

Emerging Strategies

Electroceuticals

Electric principle–based approaches are an emerging area of wound therapeutics.[17,139–147] Wireless electroceutical wound dressing inhibits *P. aeruginosa* biofilms by interfering with the quorum sensing pathway and antibiotic resistance mechanisms.[148] Preclinical porcine studies showed that wireless electroceutical wound dressing could prevent or treat *P. aeruginosa* and *A. baumannii* mixed biofilms and improve visual and functional wound healing.[17,57,149] Wireless electroceutical wound dressing was found to improve the healing impact of negative-pressure wound therapy with the need for fewer dressing changes in a limited-size clinical case series.[150] An independent group showed that wireless electroceutical wound dressing could inhibit various other pathogenic biofilm-forming bacteria in vitro.[151,152] A second-generation patterned electroceutical dressing was developed to treat deeper biofilm infections and was recently shown to be safe for human application.[153] An in vitro agar-based model using a bioluminescent strain of *P. aeruginosa* measured biofilm inhibition when direct current was applied. Scanning electron microscopic imaging identified a disrupted biofilm architecture. Mechanistically, hypochlorous acid was hypothesized to be responsible for the observed eradication of these biofilm-forming bacteria, based on pH measurement and the presence of chlorotyrosine in the cellular lysates tested.[154] Electronic scaffolds that generate hypochlorous acid have been tested for their ability to inhibit biofilm formation using an ex vivo porcine ear model, promoted by the addition of maltodextrin (a hyperosmotic agent).[155,156] Limitations

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of such study include the lack of host immune defense system as part of the experimental model. Further clinical and mechanistic studies are warranted.

Phage Therapy

The basic concept involves the use of a virus to directly lyse a bacterial cell. Phages are very specific for the bacteria being targeted and can only gain entry into a cell in response to specific receptor-mediated interactions.[157,158] Recent studies, however, indicate that phages may impact the host immune system, thereby promoting bacterial infections.[159–162] Bacteriophages have been extensively studied as therapeutic agents using ex vivo or in vivo wound models, including acute burn wound infections, alone or in conjunction with other therapeutics.[163] Phage infections can degrade biofilm matrix by inducing protease synthesis and cause whole bacterial cell lysis.[164–166] A better understanding of underlying mechanisms must be gained to pave the way toward clinical testing of this interesting therapeutic strategy.

Challenges/Closing Concept

The ideal antibiofilm strategy in the context of wound therapy would eradicate the biofilm and either promote wound closure or at least have no adverse effect on wound healing. There are antibiofilm strategies tested and marketed that appear to be effective against bacterial biofilms, but they do not fully consider all microbial (fungal, protozoan) pathogens. Furthermore, for biofilm of relevance to human health, there are two primary factors—(1) microbial mechanisms and (2) host response—that modulate microbial mechanisms over time.[57] This iterative interaction between microbes and host defenses helps shape a pathogenic chronic biofilm. Therapies marketed as "antibiofilm" may not necessarily be useful in fighting wound infections, especially if they have

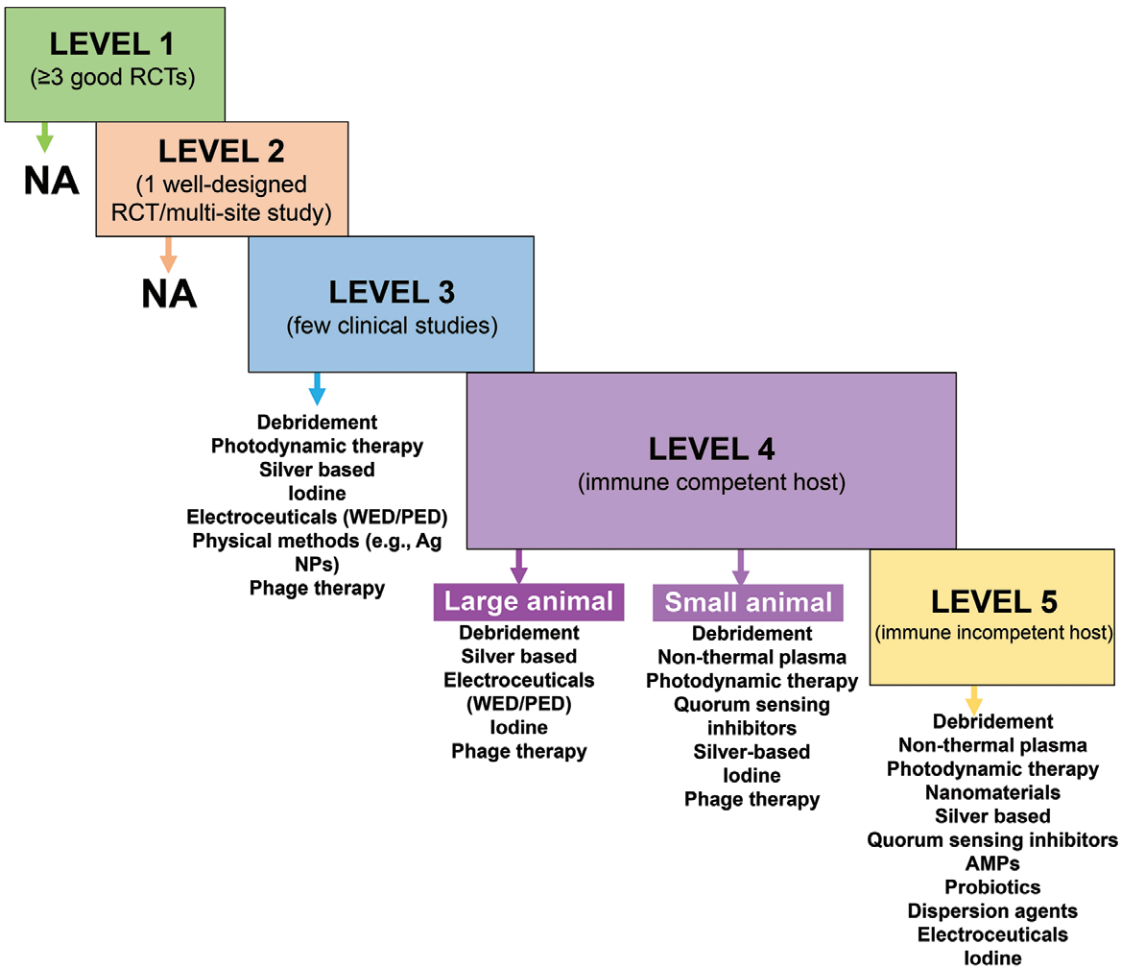

Fig. 4. Levels of evidence modified for antibiofilm strategies. *RCT*, randomized controlled trial; *NA*, not applicable; *WED*, wireless electroceutical wound dressing; *PED*, patterned electroceutical wound dressing; *AG*, silver; *NP*, nanoparticle; *AMPs*, antimicrobial peptides.

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been tested primarily in immunoincompetent (independent of host immune function) systems.[15,57] Such approaches are powerful in understanding microbiological processes but limited in addressing host-associated biofilm responses. Translational relevance of antibiofilm therapies are better tested in the context of immunocompetent preclinical models that capture the persistent nature of biofilm-infected chronic wounds.[57] Although preclinical studies ensure safety and efficacy of therapeutics, there are limitations because of the disparate anatomy and biology of different animal models compared to humans. The successful translation of antibiofilm therapies to the clinic would be better served by patient-based mechanistic and outcome studies to support definitive antibiofilm claims in wound care. A modified representation of levels of evidence in the context of antibiofilm strategies is presented in Figure 4. Most of the currently available antibiofilm strategies fall within evidence levels 3 to 5. Evidence at level 5 should be regarded as too preliminary to act on clinically. The discipline awaits level 2 evidence that would pave the way to specific U.S. Food and Drug Administration claims relevant to efficacy in managing wound biofilm infection. Products in levels 4 (large-animal) and 5 would be the most promising based on current levels of evidence.

SUMMARY

Biofilm infection is a common but unrecognized contributor to wound chronicity. It causes loss of skin barrier function and loss of evaporative water regulation. It disables the host innate immune response and weakens the extracellular matrix at the wound site. Clinicians are further challenged by the fact that bacteria in a biofilm state do not reliably grow in culture and the only way to definitively diagnose biofilm infection is through scanning electron microscopy, which is not clinically available. Challenges in biofilm detection and lack of rigorous testing in clinical trials severely limit clinical decision support. Based on the material discussed in this work, the following recommendations are made regarding clinical management of biofilm in chronic wounds. First, assume that biofilm infection is present if wound healing is stalled. Second, débridement to convert bacteria from biofilm to the planktonic state is essential to render them susceptible to treatment. Sharp débridement remains the gold standard. Noncontact methods, such as ultrasound, should be considered if pain is a limiting factor. Third, tissue specimens should be collected after débridement to increase the yield from microbiology cultures. Fourth, débridement must be followed by immediate topical antimicrobial therapy to prevent biofilm from being reestablished. Fifth, wireless electroceutical dressings have the most scientifically rigorous preclinical testing, and U.S. Food and Drug Administration–approved products of this type are available, but insurance coverage may be limited. Cadexomer iodine is an alternative product, with some evidence of biofilm eradication, and is readily available. Sixth, absorbable antibiotic-impregnated beads are effective topical antimicrobial therapy in the setting of flap closure of wounds. Seventh, topical antimicrobial therapy may not be sufficient alone, especially in the setting of underlying osteomyelitis and flap closure; thus, systemic antibiotic therapy should be included.

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