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Biofilm Management in Wound Care

Chandan K. Sen, PhD[1,2,3], **Sashwati Roy, PhD**[1,2,3], **Shomita S. Mathew-Steiner, PhD**[1,2,3], **Gayle M. Gordillo, MD**[1,2,3]

[1]Indiana University Health Comprehensive Wound Center

[2]Indiana Center for Regenerative Medicine & Engineering

[3]Indiana University School of Medicine, Indianapolis, IN, USA

Learning Objectives

After reading 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 it pertains 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 CDC estimates for human infectious diseases caused by bacteria with a biofilm phenotype is 65% and NIH estimates is closer to 80%. Biofilm 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 debridement 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 debridement 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, we critically review different categories of anti-biofilm products with emphasis on their strengths and limitations as evident from the published literature.

Corresponding author: Gayle M Gordillo MD, 545 Barnhill Drive, Emerson Hall 232, Indianapolis, IN 46202, gmgordil@iu.edu.

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Introduction

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 (EPS) 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 (surface, availability of nutrients, etc.) stimuli. It is not clear if 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 as sometimes used as controls for experimental studies. However, it is theorized that planktonic mode 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 (non-laboratory) 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). Pre-clinical studies with *Staphylococcus aureus* biofilms, have shown they produce a cytotoxin called leukocidin that kills neutrophils rendering them ineffective at clearing the biofilm(15).

Presently, wound healing endpoint is based on visual observations. Per the FDA, a wound covered by skin for at least 2 weeks at 2 consecutive visits without discharge is clinically decided 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 it’s 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 criteria for healed wounds. (17–19). Additionally, biofilm infection severely compromises the extracellular matrix composition (upregulated 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 trans-epidermal water loss (TEWL). TEWL detection devices are FDA approved for use in dermatological 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 TEWL) in over a third of all cases. Taken together, these observations lend credence to the notions that: (i) restoration of barrier function should

be factored in to a functional wound closure decision, and (ii) that TEWL readings could be used as a biomarker of wound recurrence. Currently, NIH sponsored clinical studies are in progress testing the significance of TEWL in wound care (clinicaltrials.gov NCT02577120).

Biofilm infections are a pernicious factor in human health(30, 31). The Centers for Disease Control (CDC), National Institutes of Health (NIH) and the Food and Drug Administration (FDA). Per CDC estimates, 65% of all human infectious diseases are caused by biofilm bacteria. NIH estimates that this number is closer to 80%(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 blood stream infections, orthopedic implants, urinary catheters, craniofacial and dental implants(5, 6, 32–48). It is now federally regulated that pre-market submissions of medical devices must include anti-biofilm strategies(49). The hunt for anti-biofilm solutions in healthcare has gained momentum(1, 2, 50–55). The objective of this work, is to discuss anti-biofilm strategies employed in wound care. (Video1) (See Video 1 [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 lifecycle that is targeted: **a.** adhesion inhibitors, **b.** biofilm maturation (communication) inhibitors, and **c.** promoters of disruption. To achieve these, several types of physical, chemical and biological agents/methods have been tested. None of these have been formally risen to the strength of standard of care primarily because of scanty clinical evidence. A few key agents/methods are listed in Tables 1–2. Of note, vast majority of these products have not gone through FDA clinical trials to specifically secure anti-biofilm claim. Yet in wound care education sessions at national meetings, products are presented as biofilm directed management products with minimal substantiated 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, include: i) aggregate embedded in extrapolymeric substance (EPS) matrix ii) adherence to a surface or each other; iii) persistent and localized infection; and iv) resistance to anti-microbial treatments(56). In addition, a clinically relevant model of biofilm infection must allow for host-microbe interaction under conditions of competent immune system(57, 58). Scanning electron microscopy (SEM) is currently a widely acknowledged gold standard to demonstrate polymicrobial aggregates adhered to wound surfaces and embedded in EPS. Colony forming unit (CFU) viability assays are not reliable because these assays do not account for viable but non-culturable (VBNC) 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 following ‘anti-biofilm’ strategies below in the context of these criteria and 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

(a) Debridement.

Historically, the management of invasive wound infection included systemic antimicrobial therapy in combination with debridement. While debridement can be very powerful in debulking hostile biofilm aggregates, lack of visualization of biofilm aggregates during debridement makes it a hit-or-miss type of approach that limits effectiveness. In worse case scenarios not under the control of the care provider, debridement may inadvertently push the unseen biofilm structures deeper as demonstrated in a pre-clinical studies where debridement was conducted by a plastic surgeon(18). A clinical case is presented in Figs. 2–3. Used alone debridement may not be sufficient for biofilm removal. However, in combination with other synergistic methods, it could promote chronic wound healing and decrease wound recurrence. For example, the use of resorbable antibiotic beads for aggressive antibiotic delivery to debrided pressure ulcers was found to significantly decrease (12.5% combination vs. 39.4% debridement only, $p = 0.03$) the recurrence rate of the ulcers(61). Despite its obvious shortcomings, sharp surgical debridement is still generally considered the gold standard for the management of biofilm because it disrupts the EPS and converts biofilm bacteria to planktonic bacteria susceptible to antimicrobial therapy for a brief window of time until the biofilm can be re-established(62, 63)

(b) Maggot therapy, involving the use of maggot excretions/secretions (ES) have been tested using *in vitro* and *ex vivo* studies for debridement and shown to be efficient in disrupting biofilms of various bacterial species including *P.aeruginosa* and *S.aureus* biofilms(64–66) and *Enterobacter cloacae(67)*. However, it was also documented that maggot therapy may be selective in its inhibitory effect. Studies showed that ES could enhance or promote biofilm formation of *Proteus mirabilis*(67). A clinical trial using larval debridement therapy as an anti-biofilm therapy was completed in 2018 (clinicaltrials.gov NCT02294175), but no publications are documented as yet.

(c) Ultrasound therapy (UST) involves the use of low-frequency (20–60Hz) sound waves to clean the wound and directly stimulate immune cells(68–70). UST debridement has been investigated as a supportive therapy for chronic wounds, and is hypothesized to both debride the wound and promote healing by increasing cellular activity, promoting synthesis of growth factors, promoting fibrinolysis, and disrupting biofilm(71–74). While dispersal of biofilms by UST has been studied *in vitro*(75), *in vivo* studies are limited. One study used CFU viability assay to assess the effect of UST. There was no significant decrease in bacterial count over the treatment period(76). At present it remains speculative whether UST has any impact on biofilm specific disruption *in vivo*. Interestingly, low-frequency direct contact UST was found to be effective in dispersing biofilms from metallic implant materials and making them susceptible to disinfectant treatment(77). The use of UST with microbubbles (MB) containing antimicrobial agents is emerging as the next-generation advancement to regular UST 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 (*Staphyloccus sp.*) 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, UST (with or without microbubbles) could have promising biofilm disrupting value for devices and implants.

(d) Physical methods.

While debridement is a physical method targeting a specific facet of the surgical/operative process, other physical strategies such as non-thermal plasma, photodynamic therapy and nanotechnology address aspects in the *peri-operative* realm. The mode of action (MOA) of these methods typically involve preventing adhesion or promoting dispersion and are generally applied to inanimate surfaces or objects. (i) Non-thermal plasma. Non-thermal or atmospheric cold plasma (ACP) involves the generation of photons, electrons, neutrons and protons when exposed to the constant supply of energy to a gas(82–84). The anti-biofilm effects of ACP are thought to be due to the generation of reactive oxygen (ROS) and nitrogen (RNS) species (including organic radicals). ACP has been tested *in vitro* and in a few small-animal studies as an anti-biofilm strategy(82). Clinically, ACPs 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 that may result in the development of persisters. (ii) Photodynamic therapy (PDT). PDT involves the use of a non-toxic photoactive dye (e.g., acridine orange, toluidine blue, photofrin, etc.(85)) that when exposed to light of a specific wavelength in the presence of oxygen, get 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. Due to the limited penetration capability, PDT is possibly most applicable to superficial infections. A few reports have studied the application of PDT against bacterial and fungal biofilms both *in vitro* and *in vivo*(86–90). Some unwanted side effects of PDT include increased biofilm-forming ability of *S. aureus*(91). Furthermore, PDT can cause allergic reactions and skin photosensitivity at the site of application. The application of PDT in the clinical setting for wound care requires significant testing and evaluation. (iii) Nanomaterials. The increased reactivity of nanomaterials (nanometer or submicron scale) and ease of control of its chemical and physical properties (23) has resulted in a surge of interest as a therapeutic option for treatment of biofilm infection. Examples include i. nanoparticles (NP) made of metal or metal oxide that disrupt the cell membrane directly or producing free radicals, ii. controlled and sustained site specific delivery of drugs using NPs such as liposomes or polymeric nanoparticles, iii. physical, irreversible disruption of biofilms using combination therapy such as gold nanoparticles or magnetic nanoparticles (MNPs, such as γFe2O3 maghemite or Fe3O4 magnetite nanoparticles) with near-infrared (NIR) light or alternate magnetic field (AMF), and iv. coating surfaces with NPs to prevent adhesion of bacteria and development of biofilms(92).

Chemical methods

(a) 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 (Ag+). Ag+ have shown effectivity against bacteria (including methicillin resistant *S.aureus* (MRSA) and vancomycin-resistant Enterococci (VRE)), 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 in the advent of silver nanoparticles (AgNP). AgNPs are less reactive and 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 anti-biofilm 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 Ag+ 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 (CFU) viability assay as a means to claim anti-biofilm 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 (SEM) 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 anti-biofilm capability. Coating medical devices with ionic or metallic silver has not shown much promise possibly due to inactivation by organic material such as blood(98). AgNP coated catheters did not allow the biofilm formation by a number of pathogens such *E.coli*, *Enterococcus sp*, *S.aureus*, *coagulase-negative Staphylococci* when tested *in vitro*(99). Clinical studies testing the effects of AgNP 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 AgNP coated stents and catheters in reducing or preventing biofilm infection(99–102). At high doses, AgNPs could be toxic to human cells(103). Another serious side effect is an 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 AgNP, particularly in the context of direct contact with human cells and tissues.

(b) 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. But unlike silver, is also able to inhibit mature biofilms of *Staphylococcus 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 (CI) combinations, that sequester iodine without limiting its inhibitory functions have been shown to have anti-biofilm effects and also wound healing capabilities in experimental animal models(105, 108). Rigorous *in vivo* studies and human clinical trials are warranted.

(c) Hypochlorous acid.: HOCl is known to rapidly eradicate pathogenic bacteria and is less toxic to mammalian cells than hydrogen peroxide. HOCl 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 – Dakins solution and Vashe. Conflicting evidence, based primarily on *in vitro* studies, present an unclear picture about the efficacy of these solutions against biofilms of different bacterial species exists. One study identified that HOCl was bactericidal against *Streptococcus* strains but unable to disrupt biofilm(112). Another study claimed in vivo evidence of the efficacy of HOCl against biofilm from swab samples and exudates collected from treated venous stasis ulcers. The issue with this study is that it is unclear if 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 anti-biofilm effect could be strain specific. Additional studies are required to dissect the true efficacy of this chemical agent against biofilm mode of growth. HOCl is thought to be the byproduct of electrical treatment modalities and is briefly discussed in the *Emerging strategies* section.

(d) Quorum sensing inhibitors.: Bacteria communicate to adapt their behavior collectively to their environment by a molecular phenomenon called quorum sensing (QS) that involves the synthesis and response to small molecules called auto-inducers (AIs) (114). QS drives the synthesis of virulence factors such as pyocyanin (*P.aeruginosa*), biofilm formation, and other activities(115). The inhibition of QS is called quorum quenching (QQ). Inhibitors with QQ 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, orthopedic and trauma devices(121), as a means to prevent the development of biofilm.

Natural products

Manuka honey.: Manuka honey (MH), 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 MH alone(122–126) or in combination with antibiotics(127, 128). Reports suggest a synergistic anti-biofilm effect of MH together with antibiotics(129). One report however indicated the emergence of persister strains of *P.aeruginosa* in MH treated samples(130). MH based wound dressings are available in the market and FDA 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 antibiofilm activity of MH(137, 138). Rigorous clinical studies are warranted.

Emerging strategies

(a) Electroceuticals.: Electric principle based approaches are an emerging area of wound therapeutics(17, 139–147). Wireless electroceutical wound dressing (WED) inhibits *P. aeruginosa* biofilms by interfering with the QS pathway and antibiotic resistance mechanisms(148). Preclinical porcine studies showed that WED could prevent or treat *P. aeruginosa* and *Acinetobacter baumannii* mixed biofilms and improve visual and functional wound healing(17, 57, 149). WED was found to improve the healing impact of negative pressure wound therapy (NPWT) with the need for fewer dressing changes in a limited size clinical case series(150). An independent group showed that WED could inhibit various other pathogenic biofilm forming bacteria *in vitro*(151, 152). A second generation patterned electroceutical dressing (PED) 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. SEM imaging identified a disrupted biofilm architecture. Mechanistically, hypochlorous acid (HOCl) 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). e-Scaffolds that generate HOCl have been tested for their ability to inhibit biofilm formation using an *ex vivo* porcine ear model, promoted by the addition of maltodextrin (a hyper-osmotic agent)(155, 156). Limitations of such study include the lack of host immune defense system as part of the experimental model. Further clinical and mechanistic studies are warranted.

(b) 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 towards clinical testing of this interesting therapeutic strategy.

Challenges/Closing Concept

The ideal anti-biofilm 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 anti-biofilm 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:

(**i**) microbial mechanisms, and (**ii**) host response, which modulates microbial mechanisms over time(57). This iterative interaction between microbes and host defenses helps shape a pathogenic chronic biofilm. Therapies marketed as “anti-biofilm” may not necessarily be useful in fighting wound infections especially if they have been tested primarily in *immune incompetent* (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 anti-biofilm therapies are better tested in the context of *immune competent* pre-clinical models that capture the persistent nature of biofilm infected chronic wounds(57). While pre-clinical 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 anti-biofilm claims in wound care. A modified representation of levels of evidence in the context of anti-biofilm strategies is presented in Fig. 4. Most of the currently available anti-biofilm strategies fall within levels 3–5. Evidence at level 5 should be regarded as too preliminary to act upon clinically. The discipline awaits level 2 evidence that would pave the way to specific FDA 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.

In 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 limits clinical decision support. Based on the material discussed in this work the following recommendations are made regarding clinical management of biofilm in chronic wounds: 1) assume biofilm infection is present if wound healing is stalled, 2) debridement to convert bacteria from biofilm to planktonic state is essential to render them susceptible to treatment. Sharp debridement remains the gold standard. Non-contact methods, such as ultrasound, should be considered if pain is a limiting factor. 3) Tissue specimens should be collected after debridement to increase the yield from microbiology cultures. 4) debridement must be followed by immediate topical antimicrobial therapy to prevent biofilm from being re-established. 4) Wireless electroceutical dressings have the most scientifically rigorous pre-clinical testing and FDA 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 5) Absorbable antibiotic impregnated beads are effective topical antimicrobial therapy in the setting of flap closure of wounds. 6) Topical antimicrobial therapy may not be sufficient alone, especially in the setting of underlying osteomyelitis and flap closure, so systemic antibiotic therapy should be included.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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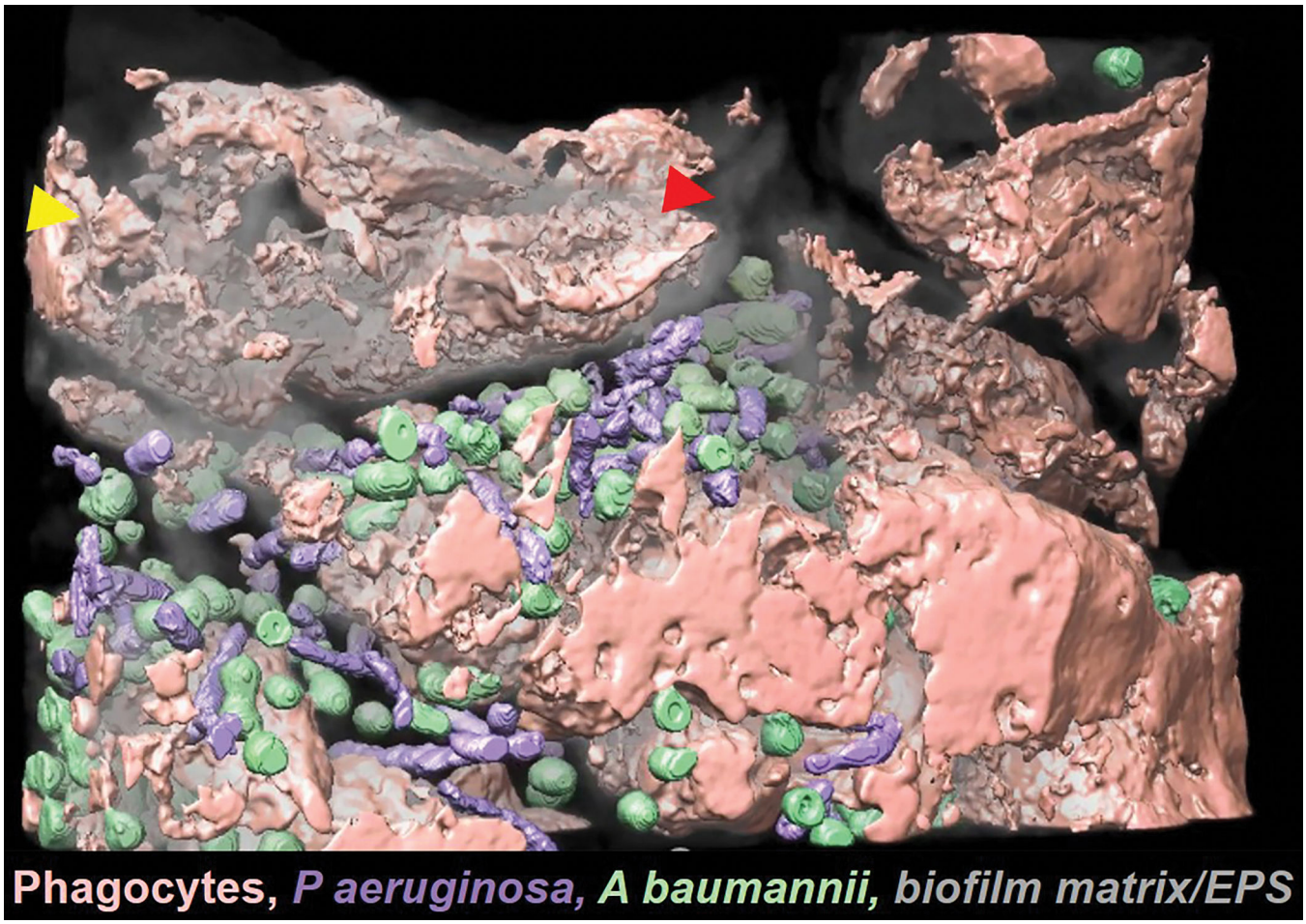

Figure 1. 3D imaging of biofilm and host immune cells.

Porcine burn wound tissue infected with Pseudomonas aeruginosa and Acinetobacter baumannii mixed species biofilm were processed and imaged using STEM/FIB-SEM imaging. Shown is a representative 3D image created from individual slices generated by the imager. Phagocytic cells are shown in pink interacting with extrapolymeric substance (EPS; grey haze, red arrow head) coated biofilm aggregates of P.aeruginosa (purple) and A.baumannii (green). Some of the phagocytic cells in this image appear to be disintegrating (yellow arrow head).

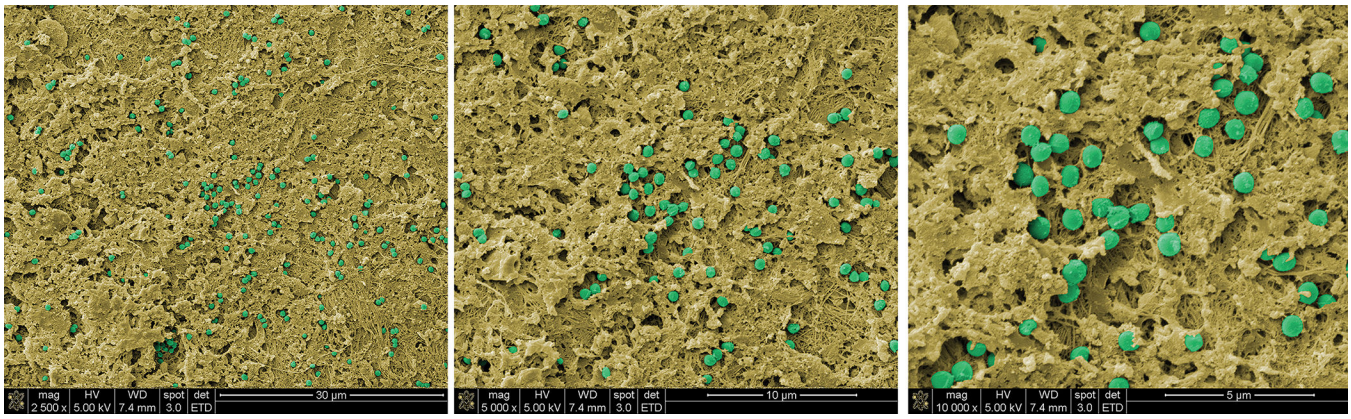

Figure 2. Aggressive tangential excision is not sufficient to eliminate biofilm infection: A case report.

A 82Y Caucasian male sustained 37% TBSA burns to his left lower extremity and posterior trunk. Upon presentation to the ED had escharotomy on left leg and was admitted to SICU for fluid resuscitation using West Penn formula. Prior to excision all burn wounds were dressed with silvadene. On post-burn day (PBD) 3 he was taken to the OR for debridement 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 post-debridement fascial wound bed was biopsied and tested positive for biofilm infection by SEM as shown. On PBD 6 he was taken back to the OR for excision and grafting of the remainder of his burn wounds on his posterior trunk and thigh. All grafts were treated with Sulfamylon soaks (5% solution). The patient had poor graft take at the site of the wound tissue biopsy with >30% graft loss. The patient developed progressive organ failure and died on PBD18.

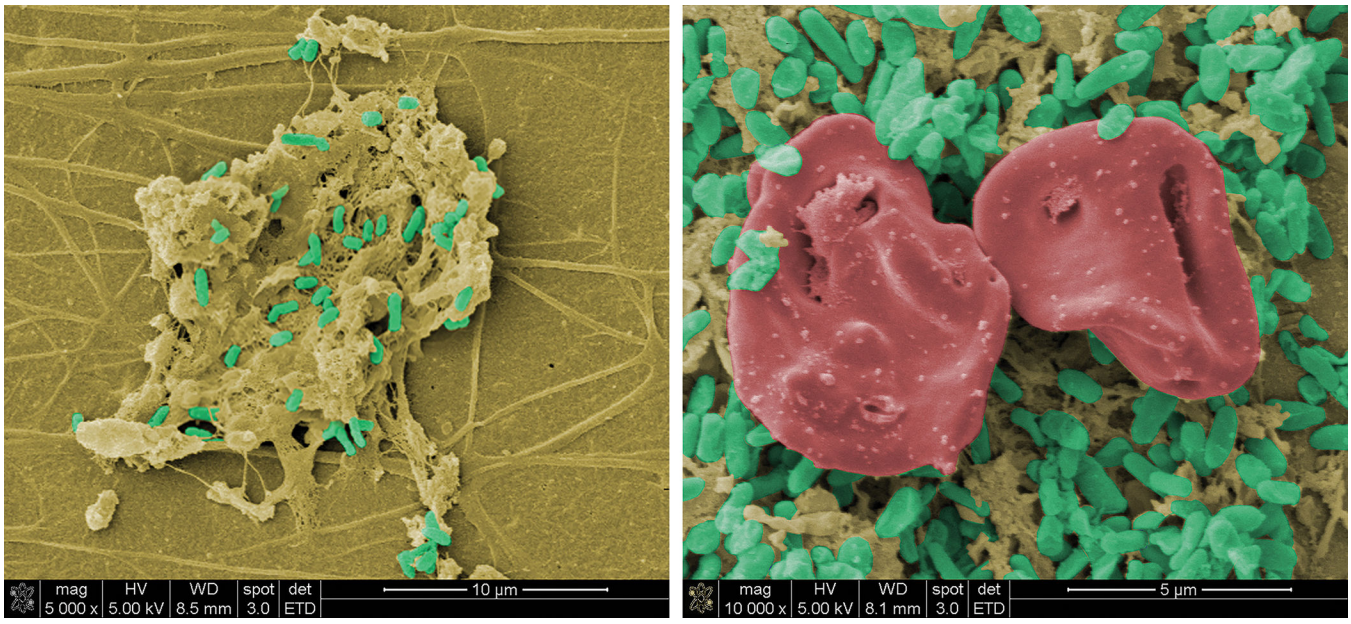

Figure 3. Biofilm in a central-line catheter taken from an in-patient with burn injury.

A. Island of biofilm cells (green) embedded in matrix (gold) in lumen of catheter. **B**. Collection of bacteria embedded in matrix surrounding red blood cells (red) in catheter tip.

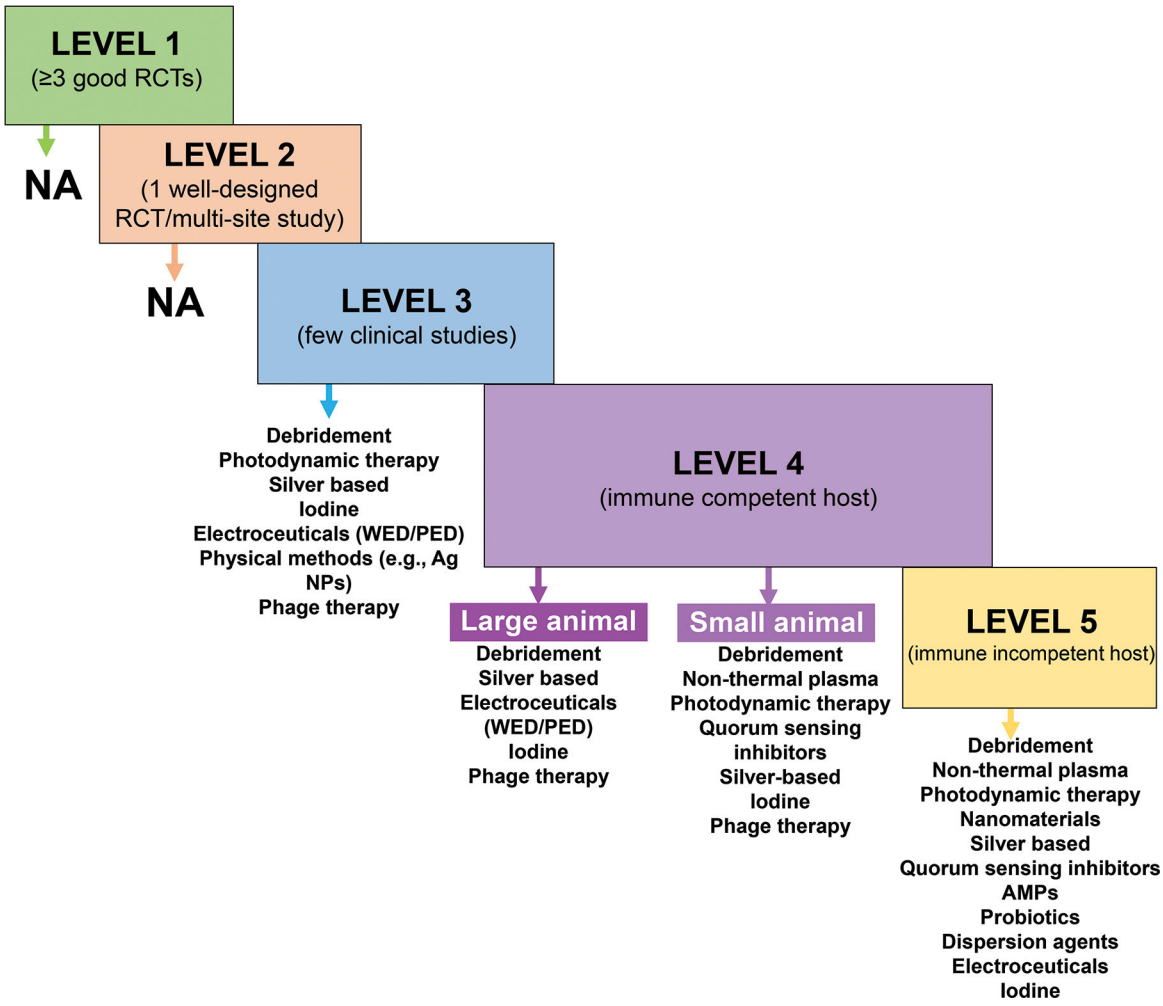

Figure 4.
Levels of Evidence modified for Anti-biofilm strategies.

Table 1.

Traditional Strategies

Strategy	Pros	Cons
Debridement	• Standard technique utilized by most surgeons • Could be used in combination with other therapies to treat biofilm	• On its own, debridement 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 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 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.

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	• Apart from WED/PED, other electrochemical approaches may not be practically used • Limited clinical studies
	• WED and PED are available in a ready-to-use dressing format that is easy to apply with minimal training. • Resistance unlikely	
Phage therapy	• Phages are easy to propagate and are highly specific for the bacterial strain targeted.	• The high specificity makes the phage a narrow spectrum application.
	• Development of resistance is low and it can be targeted to dormant and persister cells.	• 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 anti- biofilm therapy.
Antimicrobial peptides	• Broad spectrum inhibition of Gram positive and negative biofilms (IDR-1018, LL-37, DJK-5)	• AMPs are susceptible to host proteases
		• AMPs have poor bioavailability
	• Synthetic AMPs can be modified to provide additional bioactive properties (e.g., RN3).	• Expensive to synthesize
		• Insufficient clinical support via 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.