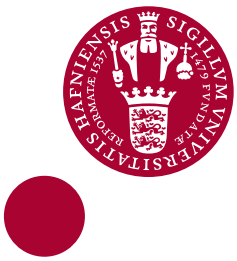

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PERSPECTIVE ARTICLE

Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds

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ABSTRACT

Background: Despite a growing consensus that biofilms contribute to a delay in the healing of chronic wounds, conflicting evidence pertaining to their identification and management can lead to uncertainty regarding treatment. This, in part, has been driven by reliance on in vitro data or animal models, which may not directly correlate to clinical evidence on the importance of biofilms. Limited data presented in human studies have further contributed to the uncertainty. Guidelines for care of chronic wounds with a focus on biofilms are needed to help aid the identification and management of biofilms, providing a clinical focus to support clinicians in improving patient care through evidence-based medicine. Methods: A Global Wound Biofilm Expert Panel, comprising 10 clinicians and researchers with expertise in laboratory and clinical aspects of biofilms, was identified and convened. A modified Delphi process, based on published scientific data and expert opinion, was used to develop consensus statements that could help identify and treat biofilms as part of the management of chronic nonhealing wounds. Using an electronic survey, panel members rated their agreement with statements about biofilm identification and treatment, and the management of chronic nonhealing wounds. Final consensus statements were agreed on in a face-to-face meeting. Results: Participants reached consensus on 61 statements in the following topic areas: understanding biofilms and the problems they cause clinicians; current diagnostic options; clinical indicators of biofilms; future options for diagnostic tests; treatment strategies; mechanical debridement; topical antiseptics; screening antibiofilm agents; and levels of evidence when choosing antibiofilm treatments. Conclusion: This consensus document attempts to clarify misunderstandings about the role of biofilms in clinical practice, and provides a basis for clinicians to recognize biofilms in chronic nonhealing wounds and manage patients optimally. A new paradigm for wound care, based on a stepped-down treatment approach, was derived from the consensus statements.

EXECUTIVE SUMMARY

A wound that is not healing in a timely fashion, despite holistic investigation and optimal intervention, can be considered as being chronic. Interventions may include treatment of infection, maintenance debridement, adequate compression (venous leg ulcers), restoration of arterial inflow (ischemic ulcers), adequate attention and intervention with respect to pressure injury (PI), offloading in diabetes-related foot ulcers (DRFUs), and management of other factors or underlying systemic diseases. After controlling for these factors, biofilms are probably

the most important single cause of persistent, delayed healing.[1–4]

Data about incidence, susceptibility, and treatment of biofilms derived from experimental animal models[5–8] and in vitro studies[9,10] have helped shape general concepts about detecting and treating biofilm bacteria, but there are limits to the extent this information can be extrapolated to clinical management. The clinical evidence base is poor, with limited human studies and few controlled trials that provide scientifically acceptable data to inform clinical management.[3,11–14] Despite publication of several treatment algorithms,[15,16] there is little published clinical data to confirm that following algorithm-guided care improves wound healing over standard care. Furthermore, there is wide disparity in clinicians' knowledge of research data that are available and the importance of biofilms in the management of chronic, nonhealing wounds.

Failure to recognize the adverse influence of biofilms can result in suboptimal treatment of chronic wounds. Current guidelines published by the European Society of Clinical Microbiology and Infectious Disease (ESCMID) Study Group for Biofilms (ESGB) have provided a strong platform for recommendations regarding the management of medically related biofilms.[3] However, these guidelines only briefly address the issue of biofilm involvement in chronic nonhealing wounds. To remedy this situation, the link from research studies to clinical practice (e.g., "bench to bedside") needs to be approached in a systematic, clear, and unbiased way. This would aid clinicians in clarifying optimal treatment strategies. An expert panel was convened in 2015 to improve understanding of best practices in wound care. The goal was to develop consensus statements for the identification and management of biofilms, drawing on the scientific literature, and the clinical and research experience of the panel members. The final consensus statements, along with the supporting evidence, are presented for 10 key topic areas. A subset of the 10 most essential (key) statements is listed in Table 1. Importantly, a new paradigm for wound care based on a stepped-down approach is presented (Figure 2).

METHODS

Panelists

A mapping process was conducted to identify panelists, who were selected on the basis of their peer-reviewed publications, scholarly activity, and reputation as an expert in chronic wounds and impact of biofilm. Diversity among panel members was sought in their geographical practices, clinical specialties, and both clinical or research activities. Ten experts (US, four; UK, two; Australia, two; Denmark, one; Japan, one) participated.

Modified Delphi process and scoring of agreement

A modified Delphi method[17] was used because it is a well-recognized and validated means of reaching

Table 1. Key consensus statements

- Wounds that contain biofilms may not be identified, resulting in ineffective treatment and delayed healing *[strong agreement, mean 4.3, SD 0.82]*.
- Biofilms are present in most chronic wounds *[strong agreement, mean 4.8, SD 0.42]*, and are likely to be located both on the surface and in deeper wound layers, but may not be present uniformly across or within the wound *[strong agreement, mean 4.5, SD 0.97]*.
- Wound biofilms are difficult to visualize macroscopically and slough, debris, and exudate may be visually mistaken for biofilm by clinicians/healthcare professionals *[strong agreement, mean 4.6, SD 0.73]*.
- Important indicators that a wound is likely to contain a biofilm include recalcitrance to treatment with antibiotics or antiseptics *[strong agreement, mean 4.3, SD 0.67]*.
- The most important measure for future diagnostic tests to consider is indication of where the biofilm is located within the wound *[strong agreement, mean 4.0, SD 0.82]*.
- Debridement is one of the most important treatment strategies against biofilms, but does not remove all biofilm, and therefore cannot be used alone—this is one of the critical principles of wound bed preparation (tissue, infection/inflammation, moisture balance, and edge of wound) *[strong agreement, mean 4.9, SD 0.32]*.
- Biofilms can reform rapidly; repeated debridement alone is unlikely to prevent biofilm regrowth; however, effective topical antiseptic application within this time-dependent window can suppress biofilm reformation *[strong agreement, mean 4.0, SD 0.67]*.
- Topical antiseptics that are effective antibiofilm treatments should have strong antibiofilm effects in appropriate in vitro test models against mature biofilms *[strong agreement, mean 4.0, SD 0.67]*.
- In vitro biofilm methods with clinically relevant test conditions are useful to screen treatments for their antibiofilm efficacy *[strong agreement, mean 4.5, SD 0.71]*.
- RCTs and comparative clinical evidence of antibiofilm treatment should be used to support clinical guidelines, protocols, and treatment choices. However, in the absence of RCT-level data, antibiofilm interventions should be supported by RCT evidence of the broader impact on wound healing *[strong agreement, mean 4.2, SD 0.79]*.

consensus on debatable clinical issues,[18] particularly when scientifically acceptable clinical evidence is lacking. To begin the process, the panel elected to address 10 topic areas considered to be important for the management of chronic nonhealing wounds: (1) problems biofilms cause clinicians, (2) understanding biofilms, (3) current diagnostic options for biofilms, (4) clinical indicators of biofilms, (5) future options for diagnostic tests, (6) biofilm treatment strategy, (7) mechanical debridement, (8) topical antiseptics, (9) screening antibiofilm agents, and (10) levels of evidence when choosing antibiofilm treatment. A detailed description of the Delphi process is presented in Supporting Information Figure S1.

A series of statements was formulated to address important aspects in each of these areas and distributed to panel members electronically (round 1 of the modified Delphi process). Regardless of statement type, panelists used their knowledge of publications in this field and/or clinical experience to guide their scoring or ranking. Statements for which consensus was not achieved were reformulated and circulated electronically and anonymously in round 2 of the modified Delphi process, along with the overall score for each statement, so each respondent could identify where their score lay in comparison. Any remaining areas where there was a lack of consensus were then addressed during extensive discussion in a face-to-face meeting at which final consensus statements were formulated and agreed upon. The final published statements listed in the text of this manuscript have, in some cases, been modified and/or consolidated by the panel after face-to-face discussion and therefore may not completely correspond to the statements from the original round of the Delphi process. Therefore, some statements do not have mean scores associated with them and are so designated by the following text: "*Full panel agreement at final meeting*." The panel agreed on a total of 61 consensus statements, grouped into 10 general topic areas. A list of key consensus statements is provided in Table 1. Statements for which consensus was not reached during the Delphi process are listed in Supporting Information Table S1.

CONSENSUS STATEMENTS

Problems biofilms cause clinicians

Key statement: (1) Wounds that contain biofilms may not be identified, resulting in ineffective treatment and delayed healing *[strong agreement, mean 4.3, SD 0.82]*.

(2) Ineffective biofilm treatment leading to delayed healing results in a decrease in patient quality of life and can put an additional burden on healthcare resources *[strong agreement, mean 4.7, SD 0.67]*.

(3) Lack of biofilm knowledge is a critical barrier to effective management of wounds that contain biofilms *[strong agreement, mean 4.0, SD 0.82]*.

(4) Ineffective biofilm treatment leading to delayed healing can put an additional burden on healthcare resources *[strong agreement, mean 4.7, SD 0.67]*.

Evidence summary

Although some indirect (surrogate) features on the surface of wound beds (e.g., extensive fibrinous slough) that are indicative of biofilm may be visible to the naked eye,[19] in many cases, the actual biofilms are located in the deeper tissue layers in the wound bed (e.g., an average depth of 50–70 microns[20]), creating issues with diagnosis. The inability to discriminate between slough and biofilm, and to reliably determine the presence of biofilm using clinical cues, may result in suboptimal care.[11] Having no definitive biomarkers or clinical cues that are available or used by clinicians compounds this further.

Furthermore, clinicians must be aware that treatment protocols based on planktonic paradigms of acute infections are not appropriate for chronic nonhealing wounds complicated by biofilms. One example of the difference between planktonic and biofilm phenotypes is the inherent tolerance of biofilms to many forms of treatment that include both systemic antibiotics and topical antiseptics.[21–28]

Understanding biofilms

Key statement: (1) Biofilms are present in most chronic wounds *[strong agreement, mean 4.8, SD 0.42]*, and are likely to be located both on the surface and in deeper wound layers, but may not be present uniformly across or within the wound *[strong agreement, mean 4.5, SD 0.97]*.

(2) The presence of biofilms and the response to them are associated with delayed wound healing *[strong agreement, mean 4.5, SD 0.71]*.

(3) Biofilms in chronic wounds are likely to be more established or mature *[strong agreement, mean 4.3, SD 0.82]*.

(4) The biofilm structure may promote the presence of anaerobic bacteria *[strong agreement, mean 4.3, SD 0.82]*.

(5) The microbiota of chronic wounds is often polymicrobial; however, wound biofilms may consist of single or multiple bacterial species *[strong agreement, mean 4.3, SD 0.82]*.

(6) Microbial diversity in a wound (planktonic and biofilms) can be influenced by location and wound characteristics *[full panel agreement at final meeting]*.

(7) Biofilms in wounds may progress to contain fewer, more dominant species over time but more research is needed to verify this *[full panel agreement at final meeting]*.

(8) Biofilms are more tolerant to the host immune response and can evade phagocytosis due to community defenses *[full panel agreement at final meeting]*.

(9) Microbial diversity in a wound can be influenced by location and wound characteristics but not necessarily wound type *[full panel agreement at final meeting]*.

Evidence summary

There is increasing evidence that biofilms are present in most, if not all, chronic nonhealing wounds.[19] A recent meta-analysis of in vivo studies highlights that at least 78% of chronic wounds contain a biofilm.[29] However, the small size and heterogeneous distribution of biofilms,[30,31] in conjunction with the finding that microorganisms in biofilms are not only located at the wound surface but may also be present in deeper tissues,[32] can explain why, when cultured, some wounds produce a false-negative result, in spite of overwhelming clinical features suggestive of wound biofilm. Figure 1 illustrates various aspects of biofilms associated with biopsies taken from chronic wounds. Although we discuss wounds as being infected with biofilm or not, in reality, it is likely that there is a ratio between planktonic and biofilm, as seen in animal models, and the relative contribution of each for any given wound can influence the efficacy of a treatment. However, if any biofilm is present, there is potential for persistent or recurrent infection.

The exact mechanisms by which biofilms can delay wound healing are still the subject of much scientific investigation. Virtually all evidence has come from either in vitro or animal models, where experimental conditions can be controlled.[32–38] Unfortunately, no large clinical studies have confirmed this. Nevertheless, biofilms have been demonstrated to play a detrimental role in many chronic diseases, such as cystic fibrosis[39–44] and sinusitis,[43–46] and may compromise in-dwelling medical devices.[47]

The contribution of biofilms to the pathogenesis of chronic wounds is shown in Supporting Information Figure S2. Biofilms are also thought to delay wound healing by eliciting an inappropriate inflammatory response, which is ineffective and poorly orchestrated, and damaging to host tissues.[16,48–51] A recent experiment in mice highlighted the ability of the microbiota (including biofilm) taken from chronic human wounds to elicit signs of chronic infection when transplanted into wounds on mice.[52]

An important contributor to the persistence of biofilms is that the biofilm matrix protects contained bacteria from systemically administered antibiotics and topical antiseptics, antibodies, complement, and phagocytosis.[53–57] Neutrophils not only have difficulty penetrating an intact biofilm: they are also unable to effectively engulf large biofilm communities. Furthermore, biofilms within a wound may vary in maturity but, once mature, biofilms are more likely to be tolerant to both antibiotics and antiseptics than biofilms still in the process of maturation.[58]

Regarding bacterial and biofilm diversity, the application of molecular techniques using DNA sequencing (amplification and sequence analysis of the 16S rRNA gene) has provided improved knowledge of the chronic wound microbiome and the microorganisms involved in biofilm infections. One such study of 2,963 patients with various chronic wounds (e.g., PI, diabetic foot ulcers, venous ulcer, and dehisced nonhealing surgical incisions) reported that Staphylococcus and Pseudomonas comprise the most prevalent genera present in the microbiota of chronic wounds, but also highlighted the high prevalence of anaerobic bacteria. Interestingly, the bacterial composition was not related to wound type.[59]

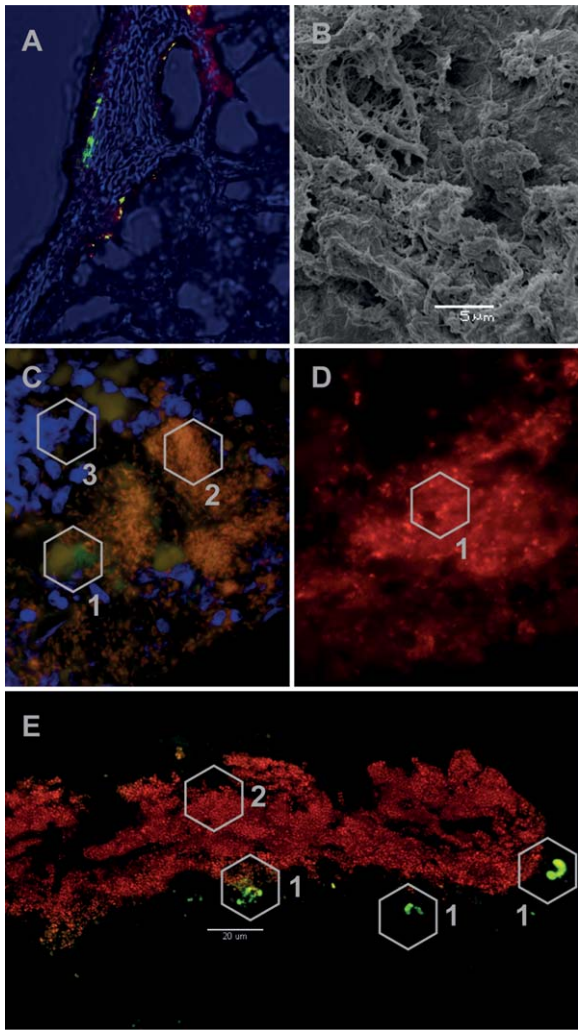

Figure 1. Microscopy images demonstrating key features of wound biofilms. (A) Peptide nucleic acid-based fluorescent in situ hybridization (PNA-FISH) analysis from diabetic foot ulcer with suspected biofilm involvement. Image courtesy of Matthew Malone. (B) Scanning electron micrograph (SEM) of chronic wound. Image courtesy of Matthew Malone. (C) PNA-FISH with confocal laser scanning microscopy (CLSM) for bacteria (eubacterial probe [green] and *Pseudomonas aeruginosa* probe [red]) surrounded by inflammatory cells (DAPI [blue]) located deep within the tissue. Image courtesy of Thomas Bjarnsholt. (D) CLSM for bacterial matrix (antibody stain against *P. aeruginosa* alginate [red, 1]) showing matrix enclosed bacteria located deep within the tissue. Image courtesy of Thomas Bjarnsholt. (E) CLSM cross-section of thick biofilm (SYTOX [green, 1]) on wound surface tissue (propidium iodide [red, 2]). Image courtesy of Randall D. Wolcott, Garth A. James, and Steven T. Fisher.

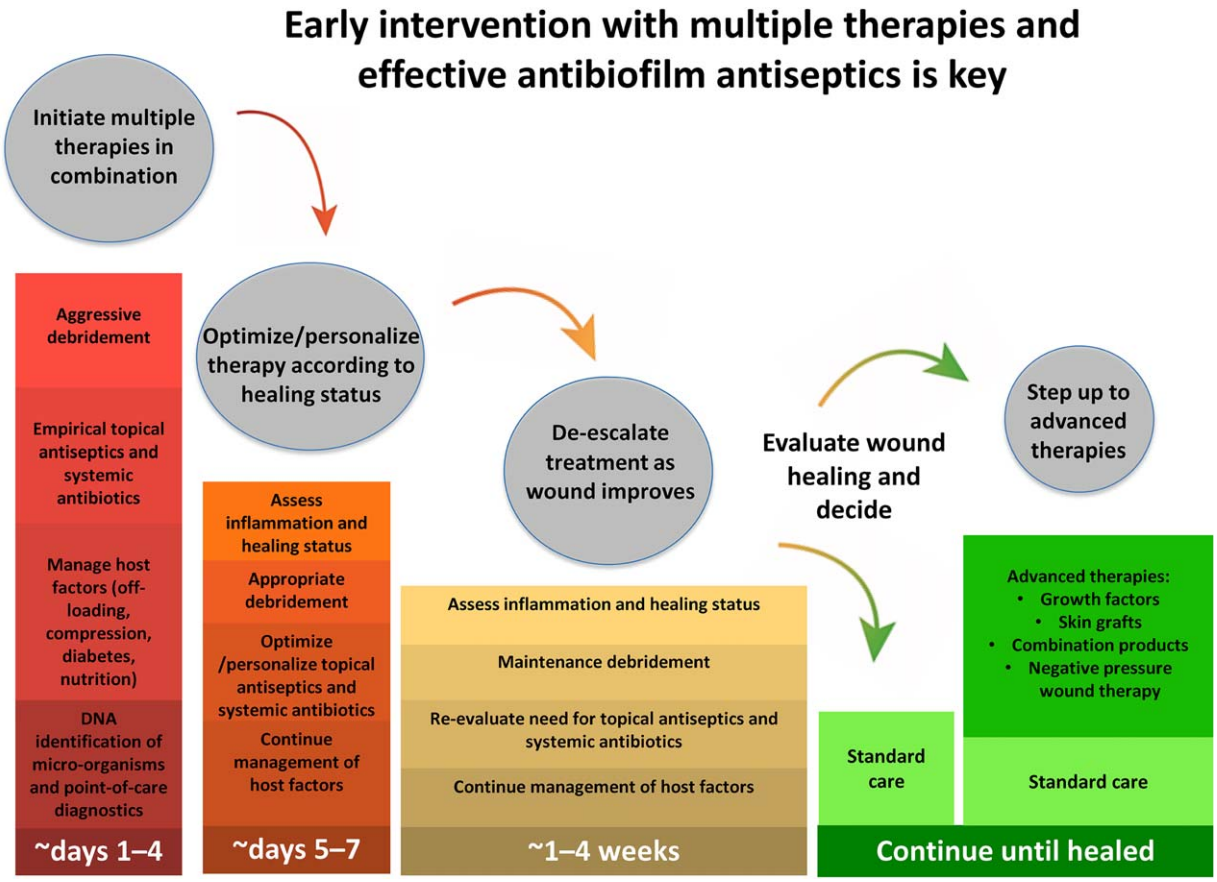

Figure 2. Summary of the step-down/step-up approach to biofilm-based wound care.

Aerobic bacteria in the biofilm, by virtue of their consumption of tissue oxygen, create a conducive environment that can promote the proliferation of anaerobes in the extracellular polymeric substances (EPS). Oxygen gradients have been demonstrated in vitro, in murine models, and in human clinical specimens.[60] The metabolism and respiratory burst of leukocytes within the wound may also deplete oxygen and, in a mouse model, *Pseudomonas aeruginosa* were shown to experience oxygen-limitation stress.[60]

Current diagnostic options for biofilms

(1) There are currently no routine diagnostic tests available to confirm biofilm presence *[strong agreement, mean 4.7, SD 0.48]*.

Key statement: (2) Wound biofilms are difficult to visualize macroscopically and slough, debris, and exudate may be visually mistaken for biofilm by clinicians/healthcare professionals *[strong agreement, mean 4.6, SD 0.73]*.

(3) Tissue biopsies are more reliable than swabs to reveal biofilms in wounds. However, routine culture techniques do not necessarily identify biofilm presence, and specialist knowledge of biofilm culture is required *[strong agreement, mean 4.2, SD 0.63]*.

(4) In the absence of bedside diagnostic tests, specific signs and symptoms should be used to confirm biofilm presence *[strong agreement, mean 4.0, SD 0.82]*.

Evidence summary

It is unlikely that aggregates in wound biofilms can be visualized with the naked eye, since they are often less than 100 μm and have no macroscopically distinguishable features.[1] Recent guidelines on medically related biofilms by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) biofilm study group (ESGB)[3] stated that approaches such as the use of scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) are the most reliable types of diagnostic techniques. SEM, for example, can identify biofilms in wounds not showing evidence of acute infection.[19] However, these imaging techniques are highly specialized and not practical in a typical clinical setting.

Sampling methods may also cause variable diagnosis; a wound swab may pick up biofilms located on the wound surface, but may fail to isolate large amounts of biofilm-

related bacteria in deeper tissues. Consequently, this method has poor sensitivity and specificity, and biofilms may be present in wounds with negative or positive culture reports.[61] Similarly, the heterogeneous distribution of biofilm across a wound means that single biopsies may also miss the biofilm during sampling[1,12,62]; however, they do sample both surface and deeper tissue. As a result, many expert groups advocate curettage samples, punch biopsies, and other samples from sharp debridement containing wound tissue as the gold standard method for identifying pathogens in biofilm infection.[63,64]

Clinical indicators of biofilms

(1) There are clinical signs and symptoms that clinicians can use to infer the presence of biofilm in a wound (even in the absence of traditional clinical signs of infection) *[weak agreement, mean 3.6, SD 1.5]*.

(2) Important indicators that a wound is likely to contain a biofilm include:

Key statement: (2.1) Recalcitrance to treatment with antibiotics or antiseptics *[strong agreement, mean 4.3, SD 0.67]*.

(2.2) Treatment failure despite using appropriate antibiotics or antiseptics *[strong agreement, mean 4.0, SD 0.67]*.

(2.3) Delayed healing *[weak agreement, mean 3.9, SD 0.99]*.

(2.4) Cycles of recurrent infection/exacerbation *[weak agreement, mean 3.8, SD 0.79]*.

(2.5) Excessive moisture and wound exudate *[weak agreement, mean 3.5, SD 1.51]*.

(2.6) Low-level chronic inflammation *[weak agreement, mean 3.4, SD 0.97]*.

(2.7) Low-level erythema *[weak agreement, mean 3.1, SD 0.74]*.

Evidence summary

It has been proposed that the presence of biofilm in wounds can be inferred by routine clinical assessments, including excessive moisture, poor quality granulation tissue, signs of local infection, history of antibiotic failure, culture-negative results despite signs of clinical infection, and failure to heal after addressing comorbidities.[15] Other authors have proposed that visual cues can be used by the clinician to guide the suspicion of the presence of wound biofilm, which can be enhanced with the use of a clinical algorithm.[19] Clinical indicators for the presence of wound biofilm presented the weakest agreement for consensus between panel members. Fibrin and slough are often mistaken for biofilm EPS and viewed as an indicator of biofilm. When exploring this concept, the panel proposes that slough is frequently a combination of plasma proteins, especially fibrin, partially degraded extracellular matrix proteins, like collagen, and devitalized tissue, which may support attachment of planktonic bacteria and subsequent biofilm development.[65] Therefore, slough should not be considered as consisting exclusively of living biofilm, but slough may often be a surrogate, "downstream" indicator of inflammation, which may be due in many cases to the presence of biofilm.

In the absence of sensitive and specific point-of-care diagnostic tests, a number of clinical features have been proposed as surrogate markers of a biofilm when a wound is not healing despite optimal standards of care. These include the failure of wounds to respond to appropriate systemic antibiotic or local antiseptic therapy in the presence of culture-guided selection; wounds that exhibit flares and quiescent periods with respect to inflammation and infection; wounds that have high levels of exudate relating to inappropriate inflammation that stimulates increased capillary permeability; gelatinous material on the wound edge that reforms quickly after removal; and mechanical curettage being needed for removal.[3,12,66]

Delayed wound healing may also be related to issues not exclusive to biofilms: sub-therapeutic dose of antibiotics; not meeting minimal inhibitory concentration due to inadequate perfusion (especially in people with ischemic limbs); repeated trauma due to inadequate offloading; incorrect dressing regimens and lack of compression therapy; and/or poor patient compliance with any current or previous culture-directed systemic antibiotic therapy. Thus, the usefulness of delayed healing as an indicator of biofilm is only likely when the "standard of care" has failed. The panel proposes that clinicians should assume that all chronic wounds that exhibit delayed healing despite optimal standards of care should be regarded as having biofilm present.

Future options for diagnostic tests

(1) The most important measures for future diagnostic tests to consider are:

(1.1) Differentiate whether the bacteria in the wound are biofilm or planktonic *[strong agreement, mean 4.6, SD 0.52]*.

(1.2) Identification of which species of bacteria are present *[strong agreement, mean 4.6, SD 0.52]*.

(1.3) Identification of the dominant species in the biofilm *[strong agreement, mean 4.0, SD 1.5]*.

Key statement: (1.4) Indication of where the biofilm is located within the wound *[strong agreement, mean 4.0, SD 0.82]*.

(2) The primary point of future diagnostics is to determine the threshold point above which wounds do not heal *[full panel agreement at final meeting]*.

(3) New tests will need clear clinical evidence and validation *[full panel agreement at final meeting]*.

Evidence summary

The heterogeneous distribution of biofilms in a wound,[58] and presence in both deeper and surface tissue,[31] poses the biggest challenge and opportunity for biofilm diagnosis. A diagnostic that could accurately locate pockets of biofilm would be advantageous to guide sampling and treatment. The search for diagnostic markers is made more difficult considering that wound biofilms can contain a wide variety of species and many are polymicrobial. Tests that detect the components of EPS can distinguish between planktonic and biofilm bacteria, but this would not necessarily identify the organisms responsible for any delay in healing. Identification of the dominant species in a biofilm may also be helpful and allow targeted therapeutics. However, research is also showing that the less dominant species may influence the pathogenicity of other species present, and therefore should not be overlooked.[67] Less prevalent species could also be more virulent and have a greater contribution to delayed healing. In addition there is some evidence to suggest that polymicrobial biofilms have higher virulence compared to single species biofilms.[68,69] The predominant microorganism(s) may also change once therapy targeting a particular species is initiated. Further challenges are recognized in that bacteria in biofilms also have an extremely low rate of metabolism, which adds to the complexity in detection compared to fast growing planktonic cultures.[70]

Biofilm treatment strategy

(1) Biofilms should be considered in the treatment of poorly healing burns *[strong agreement, mean 4.3, SD 0.67]*.

(2) Antibiofilm strategies should continue to be used until the wound bed is visibly clean, displaying healthy granulation tissue, and/or on a healing trajectory *[strong agreement, mean 4.4, SD 0.7]*.

Key statement: (3) Debridement is one of the most important treatment strategies against biofilms, but does not remove all biofilm, and therefore cannot be used alone—this is one of the critical principles of wound bed preparation (tissue, infection/inflammation, moisture balance, and edge of wound) *[strong agreement, mean 4.9, SD 0.32]*.

(4) Systemic antibiotics cannot eradicate a wound biofilm; therefore, antibiotic stewardship must be considered with controlled use to help manage planktonic bacteria, acute infection, and prevention of associated systemic infections *[full panel agreement at final meeting]*.

(5) When considering topical antiseptics, those that are known to have antibiofilm properties are preferred *[strong agreement, mean 4.6, SD 0.52]*.

(6) Prevention and treatment of biofilms should be considered in early surgical wounds when there is a high risk of surgical site infection and dehiscence (open colorectal surgery, cesarean section, and major joint replacement serve as examples) *[full panel agreement at final meeting]*.

(7) Biofilm treatments may be aligned across different types of chronic wounds, as similar dominant microflora are usually implicated—assuming the wound-specific factors are addressed with other treatment strategies *[full panel agreement at final meeting]*.

Evidence summary

All chronic nonhealing wounds in general require the same core elements of biofilm-based treatment in their management following attention and correction of associated systemic pathologies. Wound care principles should optimize wound bed preparation with respect to tissue, infection/inflammation, moisture balance, and edge-of-wound principles,[71,72] and chronic, nonhealing, wounds should be followed closely to monitor progress. These general principles have at times been conceptualized with the acronyms TIME[73] and DIME.[74,75] Additional wound-specific management should ensure that principles for different types of wounds are addressed. These include offloading for diabetic foot ulcers, pressure redistribution for PI, compression for venous leg ulcers, and adequate attention to perfusion in ischemic ulcers.

A wound cannot progress to healing when large amounts of necrotic tissue, exudate, and biofilm are present.[76] The components of biofilm, intracellular, and planktonic niches, all need to be minimized to resolve the risk of invasive infection. A clinically healing wound has "healthy" granulation tissue and a viable epithelial edge, and is visibly clean, even when minimal biofilm persists. Likewise in burn injuries, using light and electron microscopy, it has been demonstrated that biofilms may develop as early as 7 days at the site of an escharotomy, which suggests that early and complete excision is warranted.[77] (The importance of debridement is in maintaining a healthy wound bed, and is discussed further in section "Mechanical Debridement").

In vitro data suggest that there is a window of opportunity for biofilm prevention following debridement of up to 24 hours when biofilms, to the degree that they are at all susceptible, tend to have increased response to systemic antibiotics, and topical antiseptics in particular.[58] However, this is dependent on the microorganisms present and the host factors, and assumes that maintenance debridement is undertaken at dressing changes. This strategy should be used until the wound bed is visibly clean, displaying healthy granulation tissue, and/or on a healing trajectory.

In general, systemic antibiotics and topical antiseptics are of limited use in managing biofilm for several reasons as, generally, bacteria in biofilms are highly tolerant of broad classes of antibiotics and antiseptics. First, cationic antiseptics have limited penetration due to binding with anionic components in the EPS and second, bacteria in the biofilm enter a dormant-like phenotype in which there is no or little activity for antibiotics to disrupt.[78,79] In the presence of acute infection (or with masked secondary signs of infection), antibiotics should be used under the guidance of antibiotic and antiseptic stewardship guidelines.[80] These state that stewardship is defined as "coordinated interventions designed to improve and measure the appropriate use of [antibiotic] agents by promoting the selection of the optimal [antibiotic] drug regimen including dosing, duration of therapy, and route of administration."[81] The major value of systemic antibiotics is in controlling acute local infection, systemic infection, and sepsis, rather than directly treating the biofilm itself. Antibiotics should be narrow-spectrum for known sensitivities whenever possible and broad-spectrum when there is life-threatening infection/sepsis, and given usually by a parenteral route. Topical antiseptics may also help to prevent spread/reseeding of a biofilm after debridement, and their use is discussed more fully in the Topical antiseptics section. For a summary of comparative mechanisms of action of topical antimicrobials in disrupting biofilm formation, see Snyder et al. (2017).[82]

Mechanical debridement

(1) Wound bed preparation (tissue, infection/inflammation, moisture balance, and edge of wound) is an important part of biofilm treatment *[strong agreement, mean 4.2, SD 0.83]*.

(2) Surgical or conservative sharp wound debridement (CSWD) are effective ways to help remove biofilm from the surface of an open wound in which biofilm is suspected *[strong agreement, mean 4.5, SD 0.71]*.

(3) Currently, there is limited evidence for other types of debridement that include enzymatic debridement *[weak agreement, mean 3.7, SD 0.82]* or ultrasonic debridement *[weak agreement, mean 3.8, SD 0.92]*.

(4) Debridement does not necessarily remove all biofilm *[strong agreement, mean 4.6, SD 0.52]*.

(5) The removal of visible slough and debris in a wound is not sufficient to entirely remove all biofilm *[strong agreement, mean 4.8, SD 0.63]*.

Key statement: (6) Biofilms can reform rapidly; repeated debridement alone is unlikely to prevent biofilm regrowth; however, appropriate topical antiseptic application within this time-dependent window can suppress biofilm reformation *[strong agreement, mean 4.0, SD 0.67]*.

Evidence summary

Paying attention to each of the four core components of wound bed preparation is a practical guide to optimize healing of open, chronic wounds.[71–75] These principles emphasize the importance of maintaining a healthy wound bed, with debridement of necrotic tissue including biofilm, wound cleansing, and antimicrobial therapy being key components of this goal.[65,71,83]

In vivo, regrowth of mature biofilms can occur within 72 hours, but early presence of biofilms can be detected within 24 hours after debridement.[58] Repeated debridement (much like how regular tooth brushing helps control biofilm) might be able to suppress biofilm development and keep it in a weakened state so that both systemic antibiotic and topical antiseptic therapy, moisture management, and host immunity might have a better chance of suppressing the risk of infection.[76] CSWD and physical removal/disruption and prevention of re-formation of biofilms are critical to promoting healing; this is supported by several national and international guidelines.[3,11,84] The use of several modalities at one time (e.g., ultrasonic wound debridement together with CSWD using a scalpel or loop curette) may increase success; however, this has only been demonstrated in vitro.[85] Further clinical work is required to assess ultrasound for debridement and killing of bacteria associated with tissue and exudate.[7]

Although debridement can be very effective in helping to reduce bioburden and biofilms, it cannot remove all microorganisms present in the wound; in fact, one study suggests a reduction of only 1–2 logs.[86] Debridement must, therefore, be used in conjunction with topical antiseptic dressings and lavage or therapeutic irrigation to enhance further microbial reductions through killing of microbial cells and to suppress regrowth of the biofilm.[87] Removal of visible slough and debris, while useful as it might contain superficial biofilm, does not represent the total biofilm, as much will reside deeper within the wound.[51] In summary, the concepts of wound bed preparation and biofilm-based wound care emphasize the importance of combining effective wound debridement with treatments to reduce inflammation/infection by appropriate antimicrobial intervention after debridement.

Topical antiseptics

(1) In circumstances where CSWD is not possible, appropriate topical antiseptics offer an effective intervention against wound biofilms *[weak agreement, mean 3.9, SD 0.74]*.

(2) There are several reasons why some antibiotic and antiseptic treatments fail. These include:

(2.1) The slow metabolic rate of biofilm bacteria in biofilm *[strong agreement, mean 4.5, SD 0.85]*.

(2.2) Interactions with the EPS components *[strong agreement, mean 4.0, SD 0.94]*.

(3) Topical antiseptics that are effective antibiofilm treatments should:

(3.1) Reach biofilm-embedded bacteria in an active form *[strong agreement, mean 4.0, SD 1.05]*.

(3.2) Provide a high and sustained active level (due to the higher Minimum Biofilm Eradication Concentration [MBEC] required against biofilms) *[strong agreement, mean 4.3, SD 0.82]*.

Key statement: (3.3) Have strong antibiofilm effects in appropriate in vitro test models against mature biofilms *[strong agreement, mean 4.4, SD 0.7]*.

(4) Topical appropriate antiseptics are recommended as first-line therapy in stalled wounds *[full panel agreement at final meeting]*.

Evidence summary

Biofilms are extremely tolerant to multiple forms of antimicrobial treatment. There are now several in-depth reviews and scientific studies detailing the complexity of biofilm tolerance, and we will not expand upon these, but summarize the key concepts attributed to biofilm tolerance. These include: the protective layer of the EPS, which slows or inhibits the diffusion of an antibiotic into the biofilm,[11,21,88–90] and indicates that other protective mechanisms of biofilms must be at play; the concept of the "micro-niche," in which the altered chemical gradients of nutrients, oxygen, pH, and metabolites may affect both an antibiotic and or the bacteria residing in that area and retard the action of an antibiotic[60,91,92]; and the role of a very small population of specific cells within a biofilm (persister cells) that develop broad resistance against antibiotics and antiseptics.[27,93–95] The persister phenotype, dormancy, or the nutrient limitation theory can lead to inactivity of cells within the biofilm.[93,96] Due to dormant micro-niches within biofilm, antibiotics are less effective.[97] This has been highlighted by several in vitro biofilm models that have elucidated that bacteria in biofilms can withstand antibiotic concentrations considerably higher than their planktonic counterparts.[97,98] However, even with optimal kinetics and high minimal biofilm inhibitory concentration (MBIC), it is not possible to reach the concentrations needed to kill all the bacteria in biofilms in humans.[99,100]

The role of topical antiseptics in chronic nonhealing wounds has been extensively reviewed, resulting in a consensus document issued by the European Wound Management Association.[12]

Antibiotics work on specific sites in replicating (metabolically active) bacterial cells, whereas topical antiseptics generally inactivate bacteria by interaction through multiple target sites. Therefore, the use of antiseptics is less reliant on cell division for them to be effective. If the main tolerance mechanism is dormancy, and the mode of action of an antibiotic, or antiseptic, is to interfere with a cellular process, then ability to disrupt or penetrate the biofilm will not provide much of a therapeutic advantage. Appropriately used topical antiseptics are more effective after disruption of the biofilm by debridement,[101,102] and have the additional advantage of being much less likely to induce resistance compared with antibiotic treatment.[103] By delaying the re-formation of biofilm, topical antiseptics may reduce the risk of infection and the need for antibiotics, which is important in consideration of prevention of the development of antibiotic resistance.

Screening antibiotics and antiseptics

Key statement: (1) In vitro biofilm methods with clinically relevant test conditions are useful to screen treatments for their antibiofilm efficacy *[strong agreement, mean 4.5, SD 0.71]*.

(2) Test methodologies should reproducibly recover biofilm bacteria from the test system *[strong agreement, mean 4.6, SD 0.70]*.

(3) Clinically relevant in vitro methodologies should:

(3.1) Use media containing serum or blood proteins *[strong agreement, mean 4.4, SD 0.7]*.

(3.2) Use mature biofilms appropriate to laboratory design *[strong agreement, mean 4.1, SD 1.0]*.

(3.3) Include single and polymicrobial cultures relevant to the indication of interest *[strong agreement, mean 4.4, SD 0.7]*.

(3.4) Show measurable reduction in biofilm bacterial count over a clinically relevant time *[strong agreement, mean 4.2, SD 0.63]*.

(4) The use of ATP-based measurement is not considered an important way to determine biofilm viability and antibiofilm efficacy *[full panel agreement at final meeting]*.

Evidence summary

In vitro experiments offer a rapid method, and in some cases, may be the only way, to screen antibiofilm treatments.[104] Models may also provide an initial measurement of how potentially effective products may work under controlled conditions, which is important given the high complexity of in vivo human studies. As one example, a rabbit ear model has been validated and used to study numerous facets of wound healing.[5–7] However, in vitro models may not accurately represent in vivo conditions.[99] It is important that in vitro biofilm models reproduce the chronic wound environment using clinically relevant test conditions. For example, serum or blood proteins may diminish the activity of antiseptics in particular, and testing in the presence of such materials is important. The use of immature biofilms when testing antibiotics and antiseptics in laboratory experiments can lead to false success in relation to treatment efficacy. When reproduced with more mature biofilms, as would be expected in chronic wounds,

the results may not show the same efficacy.[4] Better standard methods need to be developed that are relevant to the clinical indication and environment and also type of treatment being tested.[105]

The properties of biofilms can also be influenced by the surfaces on which they are grown.[106,107] An in vitro system that more closely resembles an in vivo surface, regardless of its specific design, would be desirable, but is not absolutely necessary as long as the method can be validated. Tissue-based surfaces could conceivably allow a more natural progression of biofilm growth to occur, with potential implications on the 3D structure of a biofilm, which may be extremely important for function. However, bacteria in a chronic wound are rarely directly in contact with fresh, "healthy" tissue, so the benefits of a tissue-based growth surface are uncertain.

Test methodologies should reproducibly recover biofilm bacteria from the test system. To obtain a useful sample, some type of validated physical disruption may be necessary prior to analysis, such as sonication or repeated vortexing. This depends on the specific analysis method being used. Disruption is not necessary for molecular identification methods, as many of the DNA/RNA isolation methods already contain digestion or disruption techniques required for analysis. Molecular approaches can provide data from effectiveness studies by looking at microbial load, and diversity and presence of pathogens, in addition to microscopic methods that can look at spatial organization pre- and posttreatment, or the semiquantitative approach to visualizing biofilms through SEM or CLSM.

Any in vitro method should ideally show a measurable reduction in biofilm bacterial count over a clinically relevant time. It seems reasonable to conclude that greater log reductions are preferable, but aiming for specific log reductions may be a "planktonic concept," and not readily transferable to biofilms. In addition, quantitative criteria for infection of acute wounds may not be appropriate for nonhealing wounds.[9] It must be remembered that such high reduction in vitro may not necessarily translate to the clinical situation, and that treatments must balance antibiotic and antiseptic activity with minimal cell toxicity.

Adenosine triphosphate (ATP) has been used in laboratory situations to assess viability where a pure bacterial species is involved, but application to clinical settings is limited. In an actual wound, there are typically mixed populations of viable bacteria and wound cells also containing ATP, and dormant but viable bacteria producing less ATP. Therefore, bacterial killing cannot be assured simply by measuring ATP levels.

Levels of evidence when choosing antibiofilm treatments

Key statement: (1) Randomized controlled trials (RCTs) and comparative clinical evidence of antibiofilm treatment should be used to support clinical guidelines, protocols, and treatment choices. However, in the absence of RCT-level data, antibiofilm interventions should be supported by RCT evidence of the broader impact on wound healing *[strong agreement, mean 4.2, SD 0.79]*.

(2) Evidence from in vitro screening *[strong agreement, mean 4.0, SD 0.94]* and animal studies *[strong agreement, mean 4.1, SD 0.74]* of antibiofilm effect is important for clinicians to consider when choosing treatments, although clinical evidence is required to validate these findings.

(3) Clinical evidence from noncomparative studies and case studies is important but cannot allow for clear conclusions to be drawn on efficacy of a product *[full panel agreement at final meeting]*.

Evidence summary

Many wound treatment decisions for chronic wound management are not supported by strong evidence, and this is particularly relevant in biofilm control. Currently, the bulk of research has been conducted in vitro, with questionable relevance due to factors such as the use of "immature or young" biofilms. Validated laboratory models exist,[5–7] but they may not always be easily extrapolated to the clinical situation. Basic clinical evaluations are lacking and, where available, are commonly tested in small patient numbers without controls or clear interventions. Thus, the degree to which recommended treatment algorithms improve wound healing outcomes over standard care remains to be established. One of the current issues with animal models is that they tend to be short-term without underlying comorbidities, and do not necessarily replicate a low-grade chronic infection. Acknowledging these differences in immune function and response to infection in people can help bridge the gap between in vitro tests and clinical situations.[35–38]

Significant advances in the understanding of how antibiofilm agents work can be made with the appropriate use of in vitro systems, particularly when study designs account for relevance to a clinical scenario. If an in vitro study is able to identify an effective antibiofilm strategy, this can form the premise for undertaking more costly in vivo clinical studies. This, in turn, may help bridge the gap from bench to bedside, providing a powerful basis to aid alterations to clinical decisions. Furthermore, evidence from in vivo studies should explore whether antibiofilm agents are effective in killing/dispersing/reducing bacteria associated with biofilm compared to controls. Ideally, data should be obtained from clinical RCTs, but in vivo RCTs in this field are hard to undertake and expensive, and heterogeneity among trials is high.

CONCLUSION

This consensus document aligns existing scientific knowledge on biofilms, much of which is from in vitro studies and animal models (as opposed to human clinical trials) or the wider understanding of biofilms in other human diseases, with expert opinion on best practices for the treatment of biofilms and management of the chronic wounds with which they are associated.

The consensus statements are shaped by panel members' scientific and clinical experience, accompanied by data from published scientific communications. The panel hopes that this document provides clarity on areas of

biofilm identification and management, and can be used as a tool for clinicians seeking to gain greater understanding of biofilms and how they relate to translating research to best clinical practice. The panel's activity represents an important next step in working toward improving outcomes for patients with nonhealing wounds, and, in the future, it is our hope that these consensus statements will be supported by appropriate clinical studies.

Some algorithms have been published to try to capture the key signs and symptoms of biofilm presence.[15,16] However, there is a need for more clinical validation of such algorithms before they become truly accepted broadly into clinical practice. It is beyond the scope of this consensus document, as well as the existing evidence, to recommend specific products or therapeutics. Ideally, future studies will confirm that following specific algorithm-guided treatment results in improved clinical outcomes over standard care, and the panel hopes to prepare another document that will focus on these issues.

Based on the panel's assessment of data and recommendations, a major concept that has emerged is the need for strong initial combination treatment (i.e., the most effective debridement technique in conjunction with the most effective antibiofilm treatment) to rapidly and effectively reduce biofilm levels within wounds, and subsequently reduce inflammation, reactive oxygen species, and protease activities. This early intervention should be followed by stepped-down treatment as shown in Figure 2. The result will be more rapid healing of wounds, which reduces cost, as well as risk of amputation, and could lead to improved patient quality of life.

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Supporting Information

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