Management of wound biofilm made easy

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Introduction

The formation of biofilm occurs through an array of processes that are potentially reversible in the early stages of microbial colonisation. However, as biofilm formation progresses, disruption and eradication become increasingly difficult. In part, this is because planktonic (free-floating) microorganisms and microbial colonies that make up biofilm behave differently in terms of their protective behaviours. In addition, *in vitro* studies have shown that biofilm may have a role in inhibiting wound healing.

Authors: *Bjarnsholt T[1], Eberlein T[2], Malone M[3], Schultz G[4]*
Full author details are on page 6

The presence of biofilm may be assumed in all chronic non-healing wounds but clinical assessment should be performed to confirm the presence of it. Biofilm-based treatment should be multi-faceted with clinicians taking a step-down approach. This relies heavily on appropriate debridement and use of anti-biofilm agents that can be reduced when improvements in wound metrics are observed. Currently, there is a need for better biofilm detection methods — ideally a bedside diagnostic test — as well as evidence-based wound care protocols that help clarify debridement pathways and follow-up use of proven antimicrobial agents.

DEFINITION OF BIOFILM

Given the continued controversy and debate around biofilm and its role in the delayed healing of wounds, it is important to define the term, in order to ensure the components and specifics of biofilm-related issues are understood.

Biofilm is frequently defined based on *in vitro* observations. Classic definitions often describe biofilm as bacteria attached to surfaces, encapsulated in a self-produced extracellular matrix and tolerant to antimicrobial agents (including antibiotics and topical preparations or impregnated dressings). In addition, biofilm development is often described as multi-stage, beginning with the initial attachment of single cells to a surface, maturation of the biofilm and, lastly, dispersal of bacteria from the biofilm[1–3].

However, *in vitro* observations, based on flow cell models using glass surfaces and fresh, oxygenated culture media continuously flowing over the bacterium, differ greatly when compared to conditions within chronic wounds[4]. Here, the bacteria are not exposed to a continuous flow of fresh media and are not attached to a glass surface but, rather, to the cells of the wound bed and/or deeper tissues[5,6]. *In vivo*, chronic wound biofilm is often, but not always, encapsulated in a matrix, which contains host material, making both dispersal and treatment problematic.

Therefore, using *in vitro* observations to define, diagnose and treat biofilms in chronic wounds can be seen as misguided[7]. There are, however, commonalities between *in vitro* and *in vivo* evidence that can help in providing a definition of a biofilm. These include[8]:

- Aggregation of bacteria (where bacteria collect in numbers and stick to one another or a surface)
- A matrix of some kind that is not restricted to self-produce as it can also be of host origin
- Enhanced tolerance and protection against most antimicrobial agents and the host defence.

Based on these common criteria, a simplified description can be used to define biofilm in the context of chronic wound infection: *an aggregate of bacteria tolerant to treatment and the host defence, which is invisible to the naked eye* (Box 1)[8].

STAGES OF *IN VITRO* BIOFILM FORMATION AND REFORMATION

Based on *in vitro* observations, formation of biofilm is a multi-step process that occurs quickly and is reversible in the early stages. However, as biofilm formation progresses, disruption and eradication become increasingly difficult. Whether the biofilm formation *in vivo* follows these same steps we currently do not know. In addition, there are differences between biofilm formation on an exposed surface versus that which exists within a chronic wound.

Mythbuster: can biofilm be seen?

There has been much debate over whether biofilm, which is microscopic in nature, can be seen with the naked eye. The short answer is: no, not really. The longer answer is: it's complicated, and does not ultimately matter, given the evidence-based assumptions that can be made about biofilm, and its role in delayed wound healing.

In differing human health and disease conditions biofilm, when left to thrive, may show evidence at a macroscopic level, one example being oral plaque[9]. However, the picture is less clear for chronic wounds. Some clinicians have used rhetoric to promote what they believe are 'clinical cues' of biofilm presence, using naked-eye observations that are not based on scientific rigour[10–12]. Such signs have included a 'shiny', 'translucent', 'slimy' layer on the non-healing wound surface[11,12]; and the presence of slough or fibrin and gelatinous material that reforms quickly after disruption and removal, in contrast to slough and other devitalised tissue or fibrin, which often take longer to reform[12–14].

However, although it is arguable that these 'signs' may represent manifestations of the presence of biofilm, biofilm cannot in fact be seen with the naked eye. The new World Union of Wound Healing Societies position statement notes that 'all non-healing chronic wounds potentially harbour biofilms' and, therefore, relying on anecdotal visual cues is unnecessary[8]. Instead, clinicians should 'assume all non-healing, chronic wounds that have failed to respond to standard care have biofilm' and, therefore, treatments should be targeted towards effective disruption of biofilms and preventing their formation and reformation[8].

Furthermore, where chronic wound infections have failed to respond adequately to antimicrobial agents and standard wound care treatment, or where chronic wound infections experience periods of quiescence that alternate with acute episodes, clinical suspicion of the presence of biofilm should be raised[15]. These signs and symptoms are based on current evidence identifying that biofilm cannot be eradicated by antimicrobial agents alone, so it is fair to assume that a non-healing, chronic wound contains bacteria in the biofilm phenotype[8].

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Table 1: Stages of biofilm formation and reformation	
Stage one: reversible attachment	
	Under natural conditions most microorganisms attach to surfaces and, eventually, form biofilms[16,17]. Initial attachment is reversible. Experimental laboratory studies have shown that planktonic bacteria, e.g. *Staphylococci*, *Streptococci*, *Pseudomonas* and *Escherichia coli* typically attach (become sessile) within minutes[18,19]
Stage two: permanent surface attachment	
	Once these planktonic microbes become sessile, they form microcolonies within 2–4 hours[18,19]
Stage three: protective matrix/biofilm	
	Once firmly attached, the bacteria begin to secrete a protective surrounding matrix known as extracellular polymeric substance (EPS) and, as a result, the microcolonies become increasingly tolerant to biocides — e.g. antibiotics, antiseptics and disinfectants — within 6–12 hours[18–20]. Various secreted proteins and enzymes help the biofilm to become firmly embedded in the wound bed[18–20]
Stage four: increasing tolerance to biocides	
	Without disruption, the embedded microcolonies will evolve into fully mature biofilm colonies that are resistant to biocides — which can lead to further biofilm development — within 2–4 days, depending on the species and growth conditions[18–20]
Reformation: the window of opportunity	
	Biofilm rapidly recovers from mechanical disruption, reforming as mature, tolerant biofilm within 24–72 hours[8,18–20]. This suggests that serial wound debridement/ disruption could provide only a brief window of opportunity — less than 24 hours — in which antimicrobial treatments are at their most effective in reducing both planktonic and biofilm microorganisms in wounds[18–20]

FROM PLANKTONIC TO PROTECTION

Mechanisms of bacteria and biofilm

Microorganisms are commonly perceived to be free-floating and solitary, also known as planktonic. However, bacteria rarely present as single cells: in the air, on water, on surfaces including skin and our entire human microbiome, bacteria are present as aggregates (Figure 1a). Many different types of bacteria are commonly found on the skin of healthy people.

When these bacteria aggregate and become embedded within the wound they become sessile (immobile) (Figure 1b). In the early stages, this is reversible and the body's natural immune response can eradicate the bacteria, in particular, in acute, vascularised wounds. However, when the immune system is compromised or the effectiveness of antibiotics and wound care treatments are reduced, the resulting environment can favour development of biofilm. Immunity is affected by tissue ischaemia or necrosis, poor nutrition and/or underlying disease, for example, diabetes[13].

Once a sessile microcolony develops, important changes to the way bacteria behave take place. They begin to secrete a protective matrix known as extracellular polymeric substance (EPS)[20]. The exact composition of EPS varies according to the microorganisms present, but generally comprises polysaccharides, proteins, glycolipids and bacterial DNA — everything bacteria need to survive and propagate further (Figure 1c)[17,20,21].

In addition, bacterial DNA released by living or dead bacteria is thought to provide an important structural component for biofilm EPS matrix[22].

In vitro, mature biofilms shed planktonic bacteria, microcolonies and fragments of biofilm, which can disperse and attach to other parts of the wound bed or to other wounds, forming new biofilm colonies[1,23]. These dormant, mixed microbial communities, typical of biofilm, enable microorganisms to share their 'skills and abilities', combining their protective advantages within the EPS matrix for the survival of the group[24,25]. However, *in vivo*, the bacteria behave differently.

Traditionally, antibiotics and antimicrobials have been developed on the assumption that they would kill bacteria irrespective of where they were found. However, as most infected wounds contain slow-growing or dormant bacteria, the effect of most antibiotics is limited.

Biofilm protect the bacteria and other microbes involved, so 'protecting' the wound from treatment, maintaining it as a source of nourishment for the microcolony. Therefore, it is important to take a multi-pronged approach to disruption and eradication of biofilm, to ensure that topical antimicrobials can work optimally.

HOW DOES BIOFILM INHIBIT HEALING?

The exact mechanisms by which biofilm impairs the healing processes of wounds remain ambiguous. Current data suggest that the wound is kept in a vicious inflammatory state preventing normal wound healing cycles from occurring. The pathways behind this are not clear, but several systemic and local factors contribute to the occurrence and maintenance of a chronic wound[8]. At a systemic

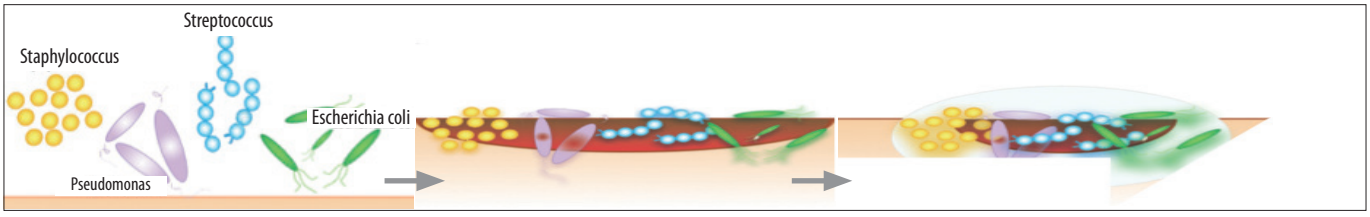

Figure 1a. Natural free-floating planktonic bacteria

Figure 1b. Initial reversible attachment

Figure 1c. Bacteria work together as a 'team' helping survival and propagation, reducing efficacy of antimicrobials

level, physiological factors include diabetes mellitus, venous insufficiency, malnutrition, malignancy, oedema, repetitive trauma to the tissue and impaired host response[8].

The majority of chronic wounds will heal if the predisposing factors are treated properly; for example, reduction of oedema in venous leg ulcers, off-loading in diabetic foot ulcers and pressure ulcers, along with the use of moist wound healing principles. At local level, biofilm inhibits healing due to its relationship with the phenotypic abnormalities of epidermis- and dermis-derived cells residing in chronic wounds, as well as the pathophysiology of a chronic wound[8].

Independent of the research on bacterial biofilm in chronic wounds, multiple laboratories have actively investigated the molecular difference between healing and chronic wounds. Among the first major molecular differences identified was the substantial elevation of two major families of proteases in chronic wounds; matrix metalloproteases (MMPs) and neutrophil elastase (NE), a member of the serine protease superfamily[26-32].

The activities of elevated protease are detrimental to healing of chronic wounds. These activities include:

- Destruction of important extracellular matrix (ECM) proteins including the multi-domain adhesion protein fibronectin[26,33], that is important in epithelial cell migration
- Destruction of important growth factors including platelet-derived growth factor (PDGF)[34]
- Degradation of key membrane receptor proteins for growth factors[35].

Similarly, proinflammatory cytokines, including tumour necrosis factor alpha (TNF-α) and interleukin-1 alpha (IL1-α), were reported as elevated in chronic wound fluid samples or biopsies when compared to healing wounds[36]. These data point to a common pathological pathway in which the development of bacterial biofilm in acute wounds stimulates chronic inflammation which, in turn, draws inflammatory cells (neutrophils, macrophages and mast cells) into the wound bed, where they secrete proteases (MMPs and NE) and release reactive oxygen species (ROS).

Development of biofilm in acute wounds leads to chronic inflammation. Elevated levels of proinflammatory cytokines lead to increased numbers of neutrophils, macrophages and mast cells that secrete proteases and ROS, which become chronically elevated and accidentally (off-target) destroy proteins that are essential for healing. The result is a chronic, non-healing wound (Figure 2)[37].

Many acute wounds can heal despite bacterial colonisation. Most wounds become chronic due to patient, host and microbe interactions. While some chronic wounds may harbour bacterial biofilm, some wounds can start to heal in the absence of antibiotics or antiseptics if patients receive timely and targeted treatment such as compression and/or offloading[8]. Why is this?

Some bacteria are more virulent (e.g. *Pseudomonas* and some *Staphylococcus* strains) than others[38], however many of the bacteria in the wounds are simply opportunistic infectious agents. It is therefore possible that the immune response might create

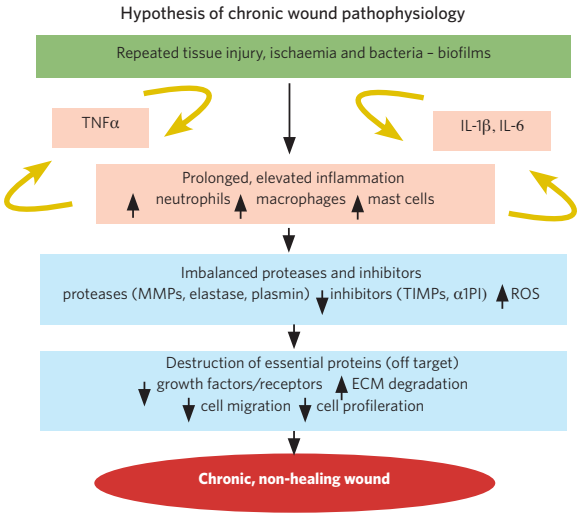

Figure 2. Hypothesis of chronic wound pathophysiology and biofilms[37]

opportunities for less virulent 'opportunist' bacteria, fighting for the same space, to influence the bacteria in the biofilm[8].

PREVALENCE AND DETECTION OF BIOFILM

Fewer than 10 studies have visualised biofilm in non-healing chronic wounds using the accepted approaches of microscopy with or without molecular analysis[5,6,39-44]. These studies identified the presence of biofilms in 60% to 100% of samples. The heterogeneity and spatial distribution of biofilm within chronic wounds and limitations of current sampling techniques in capturing tissue 'housing' biofilm means that the 'true' potential prevalence is probably closer to 100%, with all chronic wounds having biofilm on at least part of the wound bed[6,45].

Current diagnostic tests involve laboratory time, and there is no 'gold standard' test to define the presence of wound biofilm and no quantifiable biomarkers[8]. These factors may pose a significant clinical challenge given that distinguishing between planktonic or biofilm phenotype pathogenicity in chronic wound infection is a major barrier to effective treatment[8].

It is important to understand that using both culture and DNA-based methods to detect bacterial species present in wound samples does not differentiate between bacteria growing planktonically or that growing in biofilm communities[8]. This can be accomplished only by microscopy or by selective culturing for biofilms.

In May 2015, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) published guidance for diagnosis and treatment of biofilm infections[46, 47,48]. However, the guideline leaves several important questions unanswered, including whether visual signs might be useful in deciding whether to take a biopsy, where in the wound to take a sample, and whether one sample is enough[8].

TREATING BIOFILM IN CHRONIC WOUNDS

Once established within wounds, mature biofilm exhibit an enhanced tolerance to treatment. This has resulted in a paradigm shift centred on sharp debridement and adjunctive use of antimicrobial and other anti-biofilm compounds[49].

This biofilm-based wound care approach promotes a multi-faceted attack on biofilm[50] and has shown to improve the healing trajectory in a large cohort study. Implementation of personalised, topical therapeutics, guided by molecular diagnosis of bacterial species, resulted in statistically and clinically significant improvements in healing[49]. However, this does not mean that an extensive laboratory study is needed prior to beginning treatment, but rather a holistic approach to treating biofilm that considers a step-down approach to treatment.

Clinicians are encouraged to take an initial aggressive approach to treating biofilm; one that is then revised through ongoing assessment, which may result in stepping down treatment or referral to specialist services where advanced therapies may be considered if current treatment is not progressing the wound to healing. Frequent debridement is central to this step-down approach, with physical removal of microbial aggregates being key to opening up a therapeutic 'window' during which the bacteria are most susceptible to antimicrobials[50].

Clinical suspicion of biofilm

Figure 3 shows the basic principles of wound management when presence of biofilm is suspected when, the wound is:

- Failing to heal despite optimal standard care
- Not responding as expected to topical or systemic antimicrobial intervention(s)[8].

The general principles behind biofilm-based wound care and treatment strategies should include[8]:

1. Wound bed preparation

Using the TIME (tissue, infection/inflammation, moisture, edge of wound) framework is vital to assessing a wound correctly and formulating a treatment plan[51]. Sharp debridement is a key component of removing necrotic, devitalised tissue and the presence of either planktonic or sessile microorganisms. The use of topical surfactant-based wound cleansing solutions may augment the physical debridement process and are appropriate for use by wound care clinicians unable to perform sharp debridement. These surfactant-based products lower the surface tension (or interfacial tension) between a liquid and a solid and aid removal.

2. Removal of biofilm

Physical removal or attack of biofilm opens a 'window of opportunity' for increased antimicrobial susceptibility[52]. The use of antimicrobials after debridement may help to prevent biofilm reformation or aid active killing of microbial cells where residual biofilm exist. Dressings containing antimicrobials agents such as PHMB, silver, acetic acid, honey and iodine have been used against both planktonic and biofilm microorganisms to prevent reformation or as primary bactericidal agents.

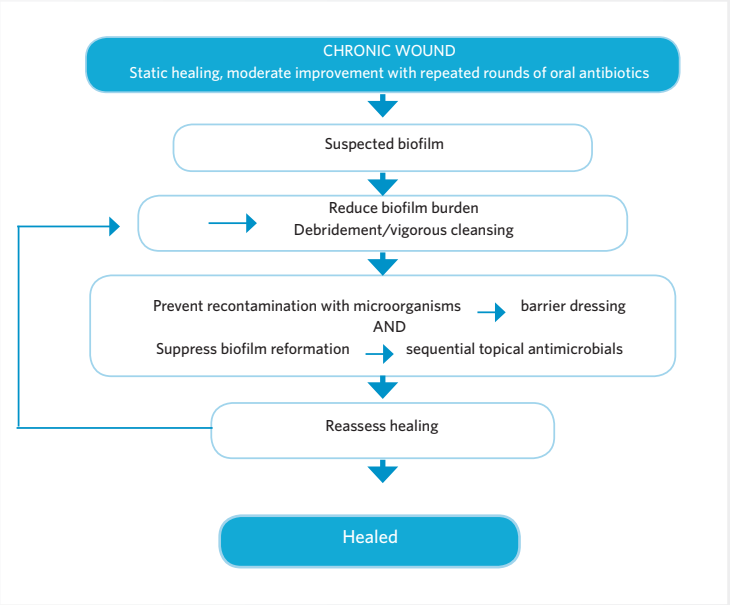

Figure 3. Principles of wound biofilm management

ANTIMICROBIALS AND 'ANTI-BIOFILM' AGENTS

To date the term 'anti-biofilm' has been synonymous with historic antimicrobials. However, clinicians should not confuse the two, as they mean very different things. Anti-biofilm agents are often (but not exclusively) novel compounds that directly influence various components of the biofilm life cycle, such as DNAse that induces dispersal. Traditionally, antimicrobials have been general, broad-spectrum, bactericidal/static agents that act on the bacteria themselves, such as the cell membranes.

The majority of topical antimicrobials used against biofilm in wound care are still traditional antimicrobials that have been tested (through various methods) against microbial cells in the biofilm phenotype and found to have an effect of sorts. The primary action of these antimicrobial agents, should they succeed, is to affect the bacteria themselves (such as cell membrane death), which may result in down-stream effects to the overall biofilm.

Much of the evidence for the action of topical antimicrobials used for various permeations of wound dressings is actually poor. All the evidence is exclusively *in vitro* and variations in testing methodologies mean that it is difficult for the results to be reproduced.

Clinicians need to be acutely aware that just because a product performs well *in vitro* does not necessarily mean it will perform well *in vivo*. A succinct review of the testing of antimicrobials against biofilm outlines some key issues for both researchers and clinicians to consider[53].

Clinicians should also be aware of the relationship between exposure time and the active delivery mechanism of many wound

dressings and solutions, and improved efficacy. The antimicrobial susceptibility of biofilm increases with exposure time[54] and clinicians should be cautious when interpreting data from *in vitro* studies of wash solutions that have reported outcomes based on 24-hour exposure time.

This is not clinically reflective; many wash solutions will be used for just seconds or minutes (most companies promote 15-minute exposure). In this scenario, it is unlikely that clinicians will see the same effects from studies reporting 24-hour exposure times.

Clinically, this means that many topical antimicrobial solutions used as irrigates or soak solutions should not be used as a sole treatment, but should form part of a multi-pronged approach that centres on sharp debridement.

FUTURE DEVELOPMENTS

Although significant progress has been made in prevention, detection and management of biofilm, more research is needed to reduce the impact on patients and on healthcare systems alike. A new, non-invasive 'biofilm wound map' technique described by Nakagami and colleagues may provide useful information on localising biofilm in the surface of a wound bed[55].
A clinician carries out a 'blot' of the wound, which is then submerged in a solution containing a dye molecule, which binds to the free bacterial DNA that partly comprises biofilm (~20%).

Researchers found that the amount of surface area of a wound bed that generated staining on the membrane predicted the extent of slough that developed on the chronic wound bed during the following week.

Box 2. Mythbuster: is biofilm 'bad'?

Is the presence of biofilm in a wound bad? The truth, to some degree, is that it depends. The presence of biofilm in the wound bed cannot be deemed beneficial when compared to there being no biofilm or virulent planktonic infection present because biofilm will almost certainly cause some level of chronic inflammation resulting in elevated proteases and ROS that impair healing[37]. The question should really be: how much biofilm can exist in a wound before causing a clinically significant delay in healing? To date, there are a little data to suggest at what level of biofilm needs to be present to negatively impact healing.

However, data are available that show that in most non-immunocompromised patients, the presence of most species of planktonic bacteria does not impair healing significantly, probably because a healthy immune system can limit the extent and spread of planktonic colonisation.

SUMMARY

Biofilm research continues to grow and evolve at a rapid pace. It is clear that researchers are still trying to understand the impact of these tolerant microbial phenotypes on wounds. More data are required particularly in the testing of both old and new agents to understand the most effective treatments. When faced with a paucity of conflicting information on biofilm, clinicians should revert to some basic principles. These include:

- Increased frequency of contact with your patient to perform aggressive (if required) debridement of the wound and general wound bed preparation. This can be reduced with improvements in wound metrics
- Disruption and subsequent removal of biofilm rarely eradicates all the biofilm in the wound and therefore should be carried out in conjunction with additional practices
- Augmented wound bed preparation with a topical wound cleansing solution that can be surfactant-based or not, but which includes a topical antimicrobial or other method to effect planktonic and/or sessile microbes
- Use of topical antimicrobials to deliver a sustained antimicrobial action following debridement and WBP
- Review/re-assess your patient frequently and monitor wound metrics
- Ensure standard of care variables are monitored closely and adhered to, e.g. compression therapy in VLUs, offloading of DFUs, re-vascualrisation where poor peripheral flow is present, etc[56]

If a wound is not progressing using the chosen treatment pathway in 4 weeks, the patient and wound should be re-assessed and an alternative regimen agreed, which may include specialist referral[56].

Author details

Bjarnsholt T[1], Eberlein T[2], Malone M[3], Schultz G[4]

1. *Costerton Biofilm Center, Department of Immunology & Microbiology, University of Copenhagen and Department of Clinical Microbiology, Rigshospitalet, Denmark*
2. *Dermatologist, Allergologist, Tissue Repair Specialist, German Wound Academy DWA, Hamburg, Germany*
3. *High Risk Foot Service, Liverpool Hospital, Sydney, Australia and Infectious Diseases and Microbiology, School of Medicine, Western Sydney University, Australia*
4. *Professor, Institute for Wound Research, Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida, USA*

A weakness of this technique is that it would preferentially detect biofilm exopolymeric matrix located on the surface of the wound bed, and not detect biofilm exopolymeric matrix buried deep in the wound bed matrix and, therefore, may not be as accurate as it should be[8].

In addition, tailored wound care protocols that help clarify debridement pathways and follow-up use of antimicrobial agents are needed. These protocols should be evidence-based while remaining flexible, so that treatment and management of all aspects of biofilm-based care can be personalised to the specific needs of the patient and the wound.

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