

Ten Top Tips...

Understanding and managing wound biofilm

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Our understanding of the factors that delay wound healing continues to improve through advances in research into the microenvironment. There is now strong evidence

that biofilm is present in the majority of chronic wounds.^[1-4] The pathogenesis of biofilms continues to be evaluated, but current knowledge suggests they are detrimental to wound healing and degrade the extracellular matrix. We acknowledge that there are gaps in the evidence and significant debate continues on how best to move the current understanding forward.

If we accept the premise that biofilm is present in the majority of chronic wounds – and that it has potential to delay healing – then the clinician requires knowledge on how to identify biofilm presence and how best to manage it. Here, the International Wound Infection Institute provides ten top tips on understanding and managing wound biofilm.

1 UNDERSTAND THE TERMINOLOGY TO GET THE MOST OUT OF RESEARCH ARTICLES AND GUIDANCE DOCUMENTS

At the most basic level, a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins. The biofilm barrier protects the microorganisms from external threats.^[5] More detailed descriptions of biofilm recognise it to be a complex microbial community that is encapsulated in an extracellular polysaccharide matrix (glycocalyx). The glycocalyx is composed of proteins, polysaccharides and extracellular DNA. The matrix of sugar and protein shields the microbial contents against the effects of the individual's immune system and many topical and systemic antimicrobial agents. The organisms within the biofilm cannot be detected using a normal wound culture method.

The following terms are key to understanding any discussion of biofilms. They are defined here specifically in the context of wound management.^[6]

Planktonic bacteria Free floating bacteria that are not attached to a wound surface. They are susceptible to systemic and topical antibiotics and can be detected using a normal wound culture swab.

Quorum Sensing The ability of bacteria to communicate with each other by releasing, sensing and responding to small signal molecules. This allows the bacteria to act like a multicellular organism with the ability to develop into biofilm and increase its defences and virulence.

Persister bacteria Quiescent (i.e. metabolically inactive) bacteria that are less susceptible to antibiotic therapies.

2 IDENTIFICATION: RECOGNISING BIOFILM IS A COMPLEX, SPECIALIST TASK

Specialised microscopic techniques used since 2008, have allowed several research groups to demonstrate that 60% to 90%^[7] of chronic wounds have biofilm formation.^[1-3,8,9]

Currently, the only definitive techniques available to detect biofilm involve advanced microscopy or specialised culture techniques. Microbiologists and researchers have used several microscopy methods to identify structures that are characteristic of biofilms such as epifluorescence microscopy, confocal laser scanning microscopy, scanning electron microscopy, and light microscopy [FIGURE 1].^[10]

As standard clinical microbiology culturing procedures only detect planktonic bacteria, special procedures must be used to culture bacteria that are present in biofilms. Typically, samples are initially treated for 24 hours in antiseptic solutions that rapidly kill all planktonic bacteria (such as brief exposure to dilute bleach) the neutralised biofilm communities are physically dispersed with ultrasonic energy and cultured on nutrient agar plates to quantitate levels of biofilm bacteria.^[11]

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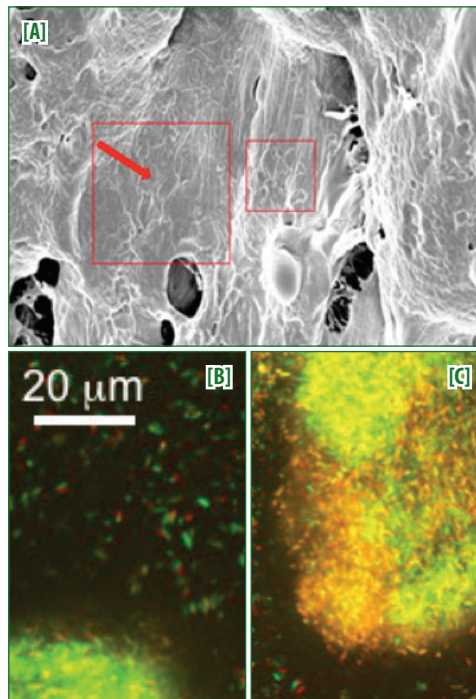
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Figure 1. Examples of *Pseudomonas aeruginosa* visualised using microscopy. [A] A scanning electron microscope shows the outlines of bacilli (red arrow) embedded in the exopolymeric matrix of a biofilm on the surface of the pig skin explant, and confocal laser scanning microscopy of *P. aeruginosa* in [B] planktonic form and [C] as part of a biofilm community.



Images courtesy of Professor Gregory Schultz.

There is significant debate as to whether clinicians can rely on clinical indicators to determine the presence of biofilm in a wound.^[10]

Table 1 summarises the key factors that may indicate the presence of biofilm. Broadly, the clinical indicators that should raise suspicion of biofilm include:

- Antibiotic failure
- Infection of >30 days' duration
- Friable granulation tissue
- A gelatinous material easily removed from wound surface that quickly rebuilds.

3 RISK FACTORS FOR BIOFILM FORMATION: BE ABLE TO RECOGNISE PATIENTS AND WOUNDS THAT ARE AT RISK

Although there is limited information regarding specific risk factors for biofilm, it is felt that many of the same factors that delay wound healing also predispose to biofilm formation.^[23]

We now understand that many medical conditions are the result of biofilm formation, cystic fibrosis, periodontitis, endocarditis, kidney stones, tonsillitis, osteomyelitis, and persistent otitis media, to name a few. Biofilms are also

associated with use of implants and prosthetics such as indwelling urinary catheters, heart valves, joint replacements and contact lenses.

Risk factors include: immuno-compromise; decreased perfusion; presence of foreign bodies; hyperglycaemia; white blood cell dysfunction; necrotic tissue; oedema; malnutrition; repeated trauma; high moisture levels. Malik et al^[24] also suggest that the following may contribute to the development of biofilm formation: diabetes, duration of ulcer >1 month, size of wound (>4 cm²), male sex, and previous antibiotic use.

4 WOUND CLEANSING: THE FIRST STEP IN REMOVING NONVIABLE DEBRIS FROM THE WOUND

Rodeheaver and Ratliff^[25] define wound cleansing as the “removal of surface contaminants, bacteria and remnants of previous dressings from the wound surface and its surrounding skin”. This definition best reflects the importance of removing all dressing product, wound debris and care of the periwound. Benefits attributed to wound cleansing are well known, but the issue appears to be when, how and, with what.

An international consensus asserts that cleansing an infected chronic wound at each dressing change is warranted.^[26] Other indicators for cleansing a wound are obvious contamination with dirt, debris, foreign matter, excess exudate, slough and nonviable tissue.

As with any wound, a holistic assessment is completed and the wound and patient requirements are determined. Optimally solutions should be at body temperature to avoid cooling of the wound and risk of slowing mitotic activity.^[27]

Methods employed for wound cleansing may vary. Therapeutic irrigation with a force of 4–15 psi has been demonstrated as effective and safe.^[28] Whatever solution is chosen to clean the wound, it should be: nontoxic; hypoallergenic; readily available; cost-effective; easy to use.

Wound cleansing solutions commonly used in wound management include: sterile normal saline, sterile water, potable tap water, and liquid antiseptics. A Cochrane review in 2008^[29] concluded that there was some evidence that using potable tap water to clean a wound may reduce planktonic bacteria; other studies suggest that normal saline and tap water are ineffective for biofilm management.^[20]

When wound infection is suspected then a solution with a surfactant, antiseptic, or antimicrobial agent is recommended. Further

investigation into the efficacy of antiseptics for anti-biofilm management is warranted, however, some commonly used antiseptic solutions are: polyhexanide (PHMB) with betaine (a surfactant); povidone-iodine; octenidine with ethylhexyl glycerine (a surfactant). As previously stated, each clinician should be aware of the cytotoxicity of each solution, appropriate concentrations and the individual wound requirements when choosing the most appropriate solution.

5 DEBRIDEMENT: MECHANICAL REMOVAL OF BIOFILM IS OFTEN REQUIRED

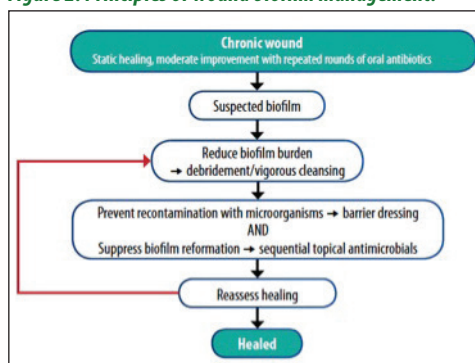
Debridement can be defined as the removal of nonviable tissue and foreign matter (including residual dressing product) from a wound. Wound bed preparation and TIME (management of Tissue, Infection and Inflammation, Moisture Balance and Edges of wound) have been considered the standard for appropriate wound management for over a decade^[30] and biofilm-based wound care incorporates these same principles [FIGURE 2].

Sharp debridement is considered the most significant method in the prevention and control of biofilm. Wolcott and colleagues^[22] have demonstrated that post-debridement biofilm is more susceptible to antimicrobial treatments for 24–48 hours. They suggest serial debridement to remove mature biofilm, followed by the application of a topical antimicrobial to address the remaining immature, more susceptible biofilm.

6 TOPICAL ANTIMICROBIALS

The action and bactericidal efficacy of topical antimicrobials against biofilm

Figure 2. Principles of wound biofilm management.^[5]



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Table 1. Clinical indicators of biofilm in chronic wounds and supporting evidence.

Excessive moisture / exudate	Evidence that excessive moisture encourages biofilm development ^[12]
Poor-quality granulation tissue (e.g. friable, hypergranulation)	High bioburden may present as friable granulation tissue ^[13]
Signs and symptoms of local infection	Secondary signs of infection are more typical of biofilm infection ^[14]
Antibiotic failure or recurring infection following antibiotic cessation	Antibiotic failure is the hallmark of biofilm infection. The use of antibiotics is still controversial regarding biofilm management; it has been suggested that – without the use of concurrent strategies for biofilm management – efficacy may be as low as 25%–30% ^[15,16]
Negative wound culture	Routine cultures will only pick up the free-floating (i.e. planktonic) bacteria, not those within a biofilm ^[17,18]
Nonhealing in spite of optimal wound management and host support	Biofilm defences include resistance to: ultraviolet light, biocides, antibiotics and host defences. Biofilm can quickly reconstitute but strategically does not kill its host ^[19]
Infection lasting >30 days	Infections of ≤30 days' duration may also contain biofilm, planktonic infection would not persist >30 days ^[15]
Responds to corticosteroids and TNF- alpha inhibitors	Inflammation is a by-product of biofilm, thus a good response to these treatments suggests presence of biofilm. Decreasing inflammation removes the primary source of nutrition ^[15]
Gelatinous material easily removed from the wound surface	Clinicians and researchers are trying to determine if the by-product of biofilm formation can be clinically seen. Case studies demonstrate differences in wound material that can be easily removed but quickly reform, either on the wound or under a dressing. Some authors believe that slough equals biofilm, but this has not been conclusively proven. A build-up of self-secreting polymers and host components is suggestive of biofilm ^[20,21]
Surface substance reform quickly	Research suggests that biofilm can reform within 24–72 hours ^[22]

“Wound care clinicians are becoming increasingly convinced that biofilms play a key role in chronic nonhealing wounds.”

bacteria have been studied *in vitro* and in a porcine skin model. In particular, both silver and iodine releasing dressings have been shown to kill biofilm bacteria.^[31–3] One study demonstrated a reduction in colony forming units over time with several silver dressings, however, cadexomer iodine achieved complete kill rates of *Staphylococcus aureus* in mature biofilms.^[33]

While antimicrobial dressings may have variable effects on bacteria in mature biofilms, they are known to be widely effective against planktonic bacteria. The best strategy for biofilm based wound care is the “clean and cover” approach, which relies on adequate debridement to disrupt biofilms and the use of antimicrobial dressings between debridements to reduce the ability of planktonic bacteria to re-establish a biofilm.

7 MOISTURE MANAGEMENT

Malik et al^[24] identified excessive moisture as a risk factor for biofilm formation. The TIME framework^[30] outlines the need to manage moisture levels with appropriate dressings or appliances. Excessive wound exudate may relate to underlying conditions including: inflammation/infection; venous insufficiency; poor compliance or concordance with compression therapy; development or deterioration of systemic causes of peripheral oedema (e.g. chronic heart failure, renal failure, liver failure); lymphoedema.

The underlying cause of excessive exudate must be determined and managed appropriately, with medical management or compression therapy should the cause be venous insufficiency or lymphoedema. Absorbent dressings should be used and the dressing change frequency adjusted to maintain a moisture balance and prevent maceration. If a biofilm is suspected, previously discussed strategies should be employed.

8 SWAB RESULTS ARE OFTEN INCONCLUSIVE; THE LEVINE METHOD IS RECOMMENDED IF SWABS ARE TAKEN

While some clinicians may infer the presence of a biofilm because of presenting clinical characteristics as previously discussed, others may choose to culture the wound. However, wound swab results may be misleading as clinical microbiology laboratories use methods that select for planktonic bacteria or are not always suitable for culture of anaerobic

species, and the sampling technique may not capture bacteria protected within a biofilm. The result is often a negative or inconclusive culture report.^[34] Methods to rapidly detect the presence of biofilm are required to assist the clinician in effective wound treatments.

Evidence suggests the best method for obtaining a wound culture of planktonic bacteria is the Levin method.^[35–7]

9 UNDERSTAND WHAT BIOFILMS REALLY MEAN FOR THE PATIENT AND THEIR WOUND

The physical barrier of the exopolysaccharide shield protects bacteria in biofilms. Furthermore, bacteria in the biofilm – especially in the periphery – can down regulate their metabolism, making them less susceptible to antibiotics. Biofilms do release antigens that stimulate the production of antibodies, but these are incapable of killing the protected sessile bacteria and instead cause damage to surrounding tissues.^[3]

Thus, biofilms are highly inflammatory, constantly shedding bacteria onto the surface of the wound, exciting an immunological response, which causes tissue damage and maintains chronic inflammation; biofilms appear to “recur” despite repeated attempts at antibiotic therapy.

10 BE AWARE OF, AND KEEP UP-TO-DATE WITH THE LATEST DEVELOPMENTS IN BIOFILM MANAGEMENT – THIS FIELD IS SET FOR FUTURE INNOVATIONS

Wound care clinicians are becoming increasingly convinced that biofilms play a key role in chronic nonhealing wounds.^[38] Even when underlying causes are managed (e.g. plantar pressure redistribution in the treatment of neuropathic diabetic foot ulcers or oedema control with appropriate compression therapy in the treatment of venous disease) many wounds are difficult to heal and exhibit continuing or reoccurring signs of infection. Future developments may include:

- Diagnostic tests to detect biofilm at the bedside
- A clearer understanding of strategies for debridement to disrupt biofilm
- Dressings that contain agents to disrupt biofilm
- Treatments that block biofilm formation through disruption of quorum sensing. ■

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