A clinical algorithm for wound biofilm identification

Recognition of the existence of biofilm in chronic wounds is increasing among wound care practitioners, and a growing body of evidence indicates that biofilm contributes significantly to wound recalcitrance. While clinical guidelines regarding the involvement of biofilm in human bacterial infections have been proposed, there remains uncertainty and lack of guidance towards biofilm presence in wounds. The intention of this report is to collate knowledge and evidence of the visual and indirect clinical indicators of wound biofilm, and propose an algorithm designed to facilitate clinical recognition of biofilm and subsequent wound management practices.

chronic wounds are defined by prevention or delay of the normal wound healing process. While underlying etiologies, such as venous insufficiency, ischaemia and hypoxia have a major impact on wound chronicity, microorganisms can also play a significant role. Recent scientific studies have identified the presence of surface-associated bacterial communities in the form of biofilm in chronic wounds. Specifically, independent research groups in the US and Europe used specialist microscopic techniques to examine debridement and biopsy samples; both groups observed biofilm microscopically in approximately 60% of all chronic wounds. Subsequent in vivo studies using a number of controlled animal models have confirmed that the presence of biofilm in wounds delays healing by interfering with granulation tissue formation, epithelialisation and host defences. Additionally, case studies and retrospective clinical studies in patients have demonstrated that biofilm-targeting treatment regimes, including sharp debridement, antibiotics, cleansers and some antimicrobial dressings, improved wound healing outcomes compared to standard care. This body of evidence now strongly supports the notion that wound biofilm plays a significant role in delaying wound healing. Moreover, it is now accepted that biofilm exists in a majority of chronic wounds, implicated in at least 80% of all human bacterial infections, and is a key risk factor for wound infection.

In the context of wound care, biofilm may be defined as communities of microorganisms attached to a surface (e.g. wound tissue, wound dressings), embedded within a hydrated matrix of extracellular polymeric substances (EPS; or slime), that provides protection against antimicrobial agents and host defenses. EPS is considered to be predominantly polysaccharide-based (although variable between bacteria), with protein, lipids and DNA also involved. In most locations where it can develop in the human body (e.g. lungs, indwelling devices, catheters) biofilm is not apparent to the naked eye, either due to its internal location, or due to its microscopic nature. However, in some instances biofilm can exist as a macroscopic and visible structure, for example as a dental plaque in individuals with poor oral hygiene. A number of clinicians have recently described what they consider to be visible biofilm in chronic wounds, in the form of translucent, slimy patches or layers, or more opaque, green-yellow substance on the wound surface. This suspected biofilm is tolerant to many antimicrobial interventions and can re-form quickly after debridement unless managed with effective antimicrobial agents and appropriate dressing technologies. Following debridement, exposed bacteria remaining in the wound can re-establish biofilm quickly, often within 24 hours, in order to evade host responses and antimicrobial therapies. This is in contrast to devitalised host tissue such as slough or fibrin which is much slower to re-form, but can itself be a sub-
strate for biofilm formation. Whereas biofilm on the wound surface may be reasonably easy to remove atraumatically using debridement methods such as swabs, fabric pads or curettage, slough is more contiguous with the underlying wound tissue and can be more difficult to remove. In addition, proteinaceous, devitalised host tissue may be more responsive to enzymatic or autolytic debridement than the complex EPS of biofilm, which may be a way to distinguish between slough and biofilm.

Identification of biofilm

Whether the material frequently observed by clinicians is actually biofilm rather than slough, fibrin or a combination of devitalised host tissue and biofilm is the subject of debate in the scientific and clinical communities. In the absence of a routine test for the presence and location of wound biofilm, this distinction is difficult to confirm. Recently, there have been calls for point-of-care wound biofilm detection methods, which, in the future may be useful to guide effective wound bed preparation and dressing selection. In our own laboratories, we have demonstrated how biofilm cultured in vitro can be rapidly and specifically stained. Using a membrane filter substrate, biofilm of a fluorescent strain of Pseudomonas aeruginosa was cultured. The biofilm was then stained with a photosensitive dye, based on dental plaque disclosing agents, that stains EPS and bacteria and fluoresces at a different wavelength to the bacterial fluorescence. This allowed imaging of the biofilm (Figure 1A) and dye location (Figure 1B) separately, by confocal laser scanning microscopy (CLSM). By overlaying these images, the intimate correlation between the biofilm bacteria, surrounding EPS and the dye was apparent (Figure 1C), confirming that

<table>
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<th>Table 1. Indirect clinical indications of wound biofilm by routine clinical assessment.</th>
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<td><strong>Clinical observation</strong></td>
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<td>Excessive moisture</td>
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<td>Poor-quality granulation tissue e.g. friable, hypergranular</td>
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<td>Signs of local infection (redness, heat, swelling, pain, odour)</td>
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<td>History of antibiotic failure or persistent or recurring infection, despite choice of antimicrobial therapy</td>
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<td>Culture-negative results despite signs of bacterial colonisation or a high suspicion of clinical infection</td>
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<td>Wound remains recalcitrant even though all obvious underlying comorbidities have also been addressed</td>
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the fluorescent dye was capable of staining biofilm. The technique of fluorescence detection offers one potential route towards a point-of-care wound biofilm detection method, perhaps using a biofilm stain and a hand-held inspection device. Such a device could be used to inspect wounds for biofilm to aid effective debridement and cleansing and to monitor wound status over time, although this might be limited to detecting surface-associated biofilm rather than any invasive, sub-surface biofilm.

However, even in the absence of a point-of-care biofilm visualisation technique, it is possible to compile clinical indicators and conditions that can facilitate judgement on the presence or absence of wound biofilm, based on current understanding of biofilm formation, composition, appearance, behaviour and treatment strategies. The intention of the current work is to consider ways in which wounds can be assessed for biofilm, and to propose a clinical algorithm that is designed to help clinicians to determine the presence of biofilm.

**Visual indicators of wound biofilm**

In some established chronic wounds, biofilm formation may progress to a stage where it is macroscopic and visible to the experienced naked eye. For example, a shiny, translucent substance on the surface of the wound (Fig 2A) may be indicative of thin but observable biofilm, careful debridement of which may reveal a healthier wound bed. At this stage, the wound may be cleansed and covered with an appropriate dressing that minimises the opportunity for remaining bacteria to re-form biofilm. Wound biofilm may also present as opaque, loosely-attached patches in some parts of the wound (Fig 2B), that can be carefully and atraumatically dislodged using standard debridement methods. Viscous, slimy or gel-like substances can form on, beneath or even within some dressings (Fig 2C), including some antimicrobial dressings (Fig 2D). In some instances, the proliferation of certain bacterial species can result in the secretion of pigments, and in the case of two common wound-associated pathogens, *Pseudomonas aerugi-
**Visual Indicators**

1. Does the surface substance detach easily and atraumatically from the underlying wound bed using physical removed techniques such as swabs, pads or sharp debridement?

2. Does the surface substance persist despite use of autolytic or enzymatic debridement?

3. Does the surface substance re-form quickly (in 1-2 days) in the absence of frequent intervention (e.g. cleansing, debridement)?

**Indirect Indicators**

4. Does the wound respond poorly to topical or systemic antibiotics?

5. Does the wound respond poorly or slowly to dressings containing antiseptic agents (e.g. silver, iodine, PHMB), including products that may control biofilm in vitro (e.g., cadoxemor iodine, nanocrystalline silver or ionic silver containing carboxymethyl cellulose dressings)?

6. Does the wound respond favourably to multi-modal strategies such as physical debridement, cleansing, and topical antimicrobial agents and dressings?

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**References**


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**A clinical algorithm**

By combining the above visual (Fig 2) and indirect indicators of wound biofilm (Table 1), an algorithm is proposed that may assist clinicians in confirming wound biofilm and subsequently taking appropriate measures to manage it (Fig 3). This algorithm poses questions around the appearance of substances in the wound and their response to wound bed preparation techniques, then considers the wound’s behaviour to antimicrobial agents. It may be started at question 1 or question 4, depending on the appearance of the wound. This algorithm may thus facilitate clinical judgement of the likely substances involved (e.g. biofilm – viable, bacteria-derived tissue; slough – non-viable, host tissue) and the predominant mode of bacterial existence (i.e. biofilm or planktonic). This algorithm is concerned with local wound factors and should be considered only once other, endogenous pathophysiological factors, e.g. hypoxia or ischaemia, and external factors, e.g. pressure or lifestyle, have been addressed and minimised. With an increasing number of affirmative answers in the algorithm,
there will be a likewise increase in the probability of wound biofilm presence and its involvement in wound recalcitrance.

**Conclusion**

Through a review of the literature and our current understanding of wound biofilm, it has been possible to collate a variety of visual indicators that may directly indicate the presence of biofilm, together with other indirect clinical indicators, which have collectively enabled the development of a clinical algorithm for wound biofilm presence. While much-needed wound biofilm research and guidance is being published at a great pace, this algorithm will hopefully facilitate clinical recognition of biofilm and subsequent optimal wound management.
Broj publiciranih referenci koje ukazuju na prisutnost biofilma u kroničnim ranama je u porastu. Sve je veći broj dokaza koji potvrđuju da biofilm značajno sudjeluje u nezacijeljivanju rane i u bakterijskim infekcijama. Iz navedenih razloga postavlja se pitanje nedostatka postupnika o dokazima prisutnosti biofilma u rani. Namjera je ovog rada specificirati vidljive dokaze i indirektno dati kliničke smjernice tretmana biofilma u rani, te predložiti određeni postupnik za olakšanje kliničkog prepoznavanja biofilma da bi se potom promijenilo ciljano liječenje vrijeda.

**Ključne riječi:** biofilm rane; prepoznavanje; postupnik; klinička procjena

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