

Biofilms: possible strategies for suppression in chronic wounds

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Summary

Biofilms can delay wound healing significantly. The aim of this article is to highlight strategies that could be used to treat chronic wounds containing biofilms. Antibiofilm agents, their modes of action and efficacy in suppressing biofilms are discussed.

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THE MICROBIAL BIOBURDEN of a chronic wound is thought to be one of the most important barriers to wound healing (White and Cutting 2006, Ryan 2007, Tan *et al* 2007). Historically, standard microbiology laboratory operating procedures in wound care studies have reported only on planktonic or free-floating microorganisms to help guide management strategies. More recently, research has highlighted that resident or sessile (attached) microorganisms, which are phenotypically and genetically different to the planktonic (free-floating) microorganisms, are compartmentalised in the chronic wound environment (Percival and Bowler 2004a, 2004b, James *et al* 2008).

A biofilm is a community of microorganisms attached to each other or to a surface and encased in an extracellular polymeric substance (EPS), with increased resistance to cellular and chemical attack. Biofilms are likely to cause a significant delay in healing and clinicians need to consider their resistance to antimicrobials if healing is to be achieved.

A misunderstanding of the role microorganisms play in chronic wound healing may result in sub-optimal management of chronic wounds and delayed healing in some acute wounds (Costerton and Stewart 2001, Costerton *et al* 2003).

This article highlights possible approaches to suppressing biofilms in chronic wounds, thus allowing the host defence mechanisms to prevail.

The biofilm concept

As 99% of microorganisms found in their native habitats persist in a biofilm state, this mode of microbial growth is applied to the wound environment (Percival and Rogers 2005, Ngo *et al* 2007, James *et al* 2008). Planktonic bacterium, coaggregates (clusters) of bacteria, or fragments of biofilm (clusters of bacteria encased within an EPS) often attach to a surface, multiply and grow (Percival *et al* 2007, Thomas 2008).

Medical biofilms Up to 80% of human infections are thought to be related to pathogenic biofilms (Anon 1997). However, clinicians have begun to appreciate that many persistent infections are caused by biofilms. Many chronic infections influenced or induced by a biofilm, for example prostatitis, endocarditis and osteomyelitis, have been found to persist indefinitely (Percival and Bowler 2004b). It seems plausible therefore to hypothesise that biofilms have a fundamental role in chronic wound infections.

Implications of biofilms As bacteria in the biofilm multiply they continually produce 'pheromones', called quorum-sensing molecules (Box 1). These are significant in the formation of a biofilm and in the development of the bacterial community. Changes in a chronic wound biofilm, induced by quorum-sensing molecules, enhance the recalcitrance of the biofilm to antimicrobials (Sauer *et al* 2002). With an array of physical and metabolic defences, the biofilm has an enhanced resistance to antimicrobials including antibiotics, antiseptics and host defence mechanisms (Costerton and Stewart 2001, Costerton *et al* 2003, Burmølle *et al* 2006, Marion *et al* 2006). Many mechanisms inherent to the biofilm,

including physical and chemical heterogeneity, interspecies co-operation, and intercellular structure, contribute to this enhanced recalcitrance to antimicrobials (Xu *et al* 2000, Fux *et al* 2005, Burmølle *et al* 2006, Shen *et al* 2006, Chang *et al* 2007).

Most microbial metabolism and bacterial divisions occur in cells located near the biofilm surface. These active bacterial cells are thought to reproduce constantly and disperse from the biofilm at a high rate. These metabolically active cells have been shown to be the most vulnerable sub-population in the biofilm to antimicrobials, particularly antibiotics and host defences (Costerton *et al* 2003). Contrary to this, microorganisms, specifically bacteria found deep in the extracellular matrix of the biofilm, have been found to be protected from external perturbations, are less metabolically active and more recalcitrant to antimicrobial management practices (Xu *et al* 2000, Lewis 2007). In addition, microbial cells found deep within the biofilm have the ability to reconstitute the community of the biofilm extremely quickly, particularly during periods of extreme stress (Lewis 2007).

Based on the above findings, the management of a biofilm community is significantly more challenging than that traditionally used in planktonic-based wound care.

Theory and practice of wound biofilms

When skin is broken a relatively 'immature' wound is formed and the primary bacterial

defence barrier is initially compromised (Niyonsaba *et al* 2006). The host's primary objective is to prevent the bacteria that have contaminated the wound from increasing in number and inducing infection. Typically, the host easily fends off potential pathogens through inflammation (proinflammatory cytokines, matrix metalloproteases, phagocytosis and degranulation of neutrophils). However, a number of host factors promote the establishment of a chronic wound and infection, including poor perfusion, malnutrition, foreign body presence, pressure, repetitive trauma, hyperglycaemia and white blood cell dysfunction.

If a biofilm becomes established in a wound, it will be difficult to suppress, particularly in an immunocompromised individual (Costerton *et al* 2003). Consequently, the microorganisms and their extracellular components within the biofilm will prolong the state of acute inflammation indefinitely, delaying the normal healing process.

Clinicians often focus on the number of culturable bacteria that are present in the wound as this frequently correlates with the degree of immune stimulation (classic signs of acute infection) seen in the patient (Dow 2001). However, many clinical biofilm bacteria cannot be cultured and so these bacteria can often be overlooked using traditional microbiological techniques (Costerton *et al* 2003).

Sharp debridement of devitalised tissue promotes healing by removing tissue that not only supports microbial proliferation (the biofilm) but also reduces the efficacy of topical therapies. Consequently, debridement should be performed at weekly intervals in chronic wounds containing devitalised tissue and biofilm (Wolcott *et al* 2009), although this should only be carried out by those who are qualified to carry out this skill. Debridement is thought not only to remove microorganisms but also to expose deeper host defences, therefore enhancing their efficacy. However, debridement alone in the authors' opinion is not sufficient to manage the majority of chronic wounds because of the nature of biofilm removal and re-establishment. Other concomitant strategies such as antimicrobials should be considered as an adjunct therapy (Schultz *et al* 2004).

Some studies have shown that antimicrobial agents sometimes work effectively to suppress the metabolically active cells in a biofilm (Flemming *et al* 2009). However, it is important to acknowledge that no single strategy has yet proven to be consistently effective at suppressing the entire biofilm. For example, infection often reoccurs following the administration of a course of antibiotics. This is because antibiotics

BOX 1

Glossary

- ▶ **Colonisation** – the presence of bacteria in increasing numbers in the wound without inducing a host reaction. It is not possible to differentiate clinically between contamination and colonisation.
- ▶ **Contamination** – the presence of bacteria in a wound without inducing a host reaction.
- ▶ **Matrix metalloproteases** – proteases that may be endogenous or exogenous in origin and break down protein, for example collagen.
- ▶ **Planktonic** – free-floating microorganisms not attached to a surface.
- ▶ **Proinflammatory cytokines** – protein molecules derived from the immune system that amplify the inflammatory response.
- ▶ **Quorum sensing** – measuring the bacterial population concentration through the expression of bacterial signalling molecules. When a 'quorum' is reached, specific biological activities, for example expression of virulence factors are activated.
- ▶ **Sessile** – microorganisms attached to a surface.

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are only effective in transiently suppressing rapidly growing cells, which are generally located on the outermost surface of the biofilm. Recalcitrant microbial cells found deep in the biofilm will persist.

Shortcomings exist in the use of single or sequential treatment strategies. In the authors' experience, concurrent management strategies will increase the likelihood of prolonging suppression of the biofilm. Such extensive suppression of the biofilm is essential to foster sufficient healing (Costerton *et al* 2003). There are no visible clinical signs to indicate the presence of a biofilm, however if other causes of delayed wound healing such as smoking and poor nutrition have been ruled out, then a chronic infection (biofilm) is usually suspected.

Management strategies

Many commercially available wound dressings are not inherently antimicrobial. However, some

wound dressings have been shown to help reduce the bacterial load at the wound surface through sequestration (binding) of bacteria (Mertz and Eaglstein 1984, White *et al* 2006). Consequently, wound dressings should be selected carefully to help reduce the risk of biofilm growth and therefore further proliferation on and within the wound.

The modes of action of various antimicrobial agents differ. However, their effects are similar, in that they impair the metabolism or integrity of microorganisms by stopping or substantially reducing cell division (microbiostatic) or by killing microorganisms directly (microbiocidal). The most recently designed antibiofilm agents often work without impairing microbial growth, reproduction or cell integrity but by breaking up the biofilm, removing essential nutrients or metal ions, or interfering with microbial community interactions such as quorum sensing (Singh *et al* 2002, Costerton *et al* 2003, Kaneko *et al* 2007). These agents are not yet available as they are experimental developments. Once approved by the regulatory authorities they will provide a more effective approach to wound biofilm management. However, they will not be a 'one dose' or 'one application' only approach but will need to be repeated or sustained in line with current antibiotic and antiseptic use.

Systemic antibiotic treatment is used in chronic wounds in situations where there is significant deep tissue wound infection or a risk of septicaemia. However, systemic antibiotics have been shown to have a low efficacy rate when biofilms are present (Moss *et al* 1990, Marr *et al* 1997). The use of antibiotics is problematic in ischaemic wounds. This is because the therapeutic concentrations that are routinely used may not reach the site of infection at the therapeutic dose. However, systemic antibiotics have been shown to contribute to the clinical management regimen of many wound biofilms by suppressing the cells at the outermost region of the biofilm (Xu *et al* 2000).

Topical antiseptics can also help to reduce the wound bioburden and biofilm, particularly where there is a concurrent infection or an increased risk of infection.

In the authors' opinion, the efficacy of antimicrobial agents can be substantially enhanced when combined with other management strategies, specifically debridement in conjunction with an appropriate wound dressing.

Antimicrobial agents It is plausible to suggest that antimicrobial agents could act prophylactically to suppress biofilm development. However, some non-selective antimicrobials may be detrimental to wound healing by harming the host's cells, for example alcohols, hydrogen peroxide, carbolic acid,

TABLE 1

Potential antibiofilm agents		
Agent	Mode of action	Usage
Lactoferrin	Blocks the attachment of planktonic bacteria to a surface. Blocking the initial attachment of bacteria stops the first step in biofilm formation (Singh <i>et al</i> 2002)	Bovine lactoferrin is a protein used in the meatpacking industry to protect exposed meat from bacterial biofilm formation
Xylitol	Interferes with biofilm formation (Katsuyama <i>et al</i> 2005)	Xylitol is a five-carbon alcohol sugar used in chewing gum. It has been shown to reduce the incidence of dental caries (Burt 2006)
Gallium	Interferes with bacterial iron metabolism pathways (Kaneko <i>et al</i> 2007)	Gallium nitrate is approved by the Food and Drug Administration and can be used intravenously
Dispersin B	Targets the extracellular polymeric substance of some types of biofilm and works to degrade the community structure of the biofilm (Itoh <i>et al</i> 2005)	Dispersin B is a bacterial enzyme but is not presently used commercially
Honey	Possesses antibacterial activity (Molan 1999, 2006) and modulates monocytic cell activity (Tonks <i>et al</i> 2001, 2003)	Honey has shown some potential against <i>in vitro</i> biofilms (Okhiria <i>et al</i> 2004)

sodium hypochlorite and acetic acid. Selective antimicrobials such as molecular iodine and ionic silver are more suitable for chronic wounds. **Iodine** Iodine has been used for many years as a wound antiseptic (Cooper 2007). However, high doses of iodine can be detrimental to host healing (Kramer 1999, Wilson *et al* 2005). Cadexomer iodine can be used to suppress biofilms without causing significant host cell damage (Akiyama *et al* 2004). **Ionic silver** Ionic silver is a beneficial antimicrobial for use in wound care, particularly for biofilm-based management strategies. Ionic silver has a broad range of efficacy against many microorganisms (Russell and Hugo 1994, Lansdown *et al* 1997). In addition, a number of silver dressings have been shown to prevent biofilm formation

in vitro (Percival *et al* 2007) and also have *in vivo* benefits (Fong and Wood 2006). **Honey** *In vitro* evidence shows that honey is effective against a range of multi-resistant organisms including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and multi-resistant Gram-negative organisms including *Pseudomonas aeruginosa* (George and Cutting 2007), and can surpass the use of antibiotics or antiseptics in previously unresponsive wounds (Dunford *et al* 2000). Irish *et al* (2006) demonstrated the effectiveness of honey in preventing biofilm formation. Okhiria *et al* (2004) found disruption of pseudomonal biofilms (*in vitro*) following application of honey. Honey also has potential as an antibiofilm agent (Table 1). **Antibiofilm agents** The use of antibiofilm agents are considered by many to be beneficial to wound management as they have been shown to be less cytotoxic than many traditional antimicrobials. These include

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lactoferrin (Singh *et al* 2002), xylitol (Katsuyama *et al* 2005), gallium (Kaneko *et al* 2007) and Dispersin B (Lu and Collins 2007). Evidence regarding the benefits of such agents to wound healing is slowly beginning to gain momentum. Agents that have been used as antibiofilm agents in wound care can be found in Table 1. These agents are still being evaluated and are not yet available in clinical practice.

Conclusion

The aim for the wound care provider is to establish the best management strategies possible for each individual patient. To achieve this, clinicians need to be familiar with antimicrobial and antibiofilm agents. The basis of managing biofilms is frequent removal of the biofilm from the surface of the

wound. This can be achieved with either sharp debridement (curette), or formal surgical debridement. As biofilms reconstitute quickly following invasive debridement, suppressing their regrowth and reconstitution using multiple antimicrobial management strategies is warranted, including wound cleansers, topical antimicrobials and advanced wound dressings. Such strategies may work synergistically to help suppress and reduce the regrowth of the biofilm. A 'rotating' regimen of selective antimicrobials may be advantageous in biofilm-based wound management. Systemic antibiotics may also help to suppress further the biofilm.

For positive clinical outcomes it is important that all concurrent barriers to healing should be addressed. This will help to augment the host's defences which, when working optimally, provide the best strategies to help manage a wound. Consequently, biofilm-based management is becoming fundamental to non-healing wounds **NS**

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