Biofilms in wounds: management strategies

Biofilms probably induce a chronic and/or 'quiet' inflammation in the chronic wound and so delay healing. This paper reviews current strategies that can be used to suppress biofilms in chronic wounds until better options are available

antibiofilm strategies; biofilm communities; debridement; wound dressings

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Bioburden on the wound bed may be one of the most important barriers to wound healing.[1] The bioburden comprises devitalised tissue, proteinaceous exudate, effete white blood cells and, most specifically, microorganisms.[2-4] Given that surface-associated bacteria organise into biofilm,[5-7] it would appear that they may be the most important component of the wound bioburden.[8]

Research on wound bacteria has traditionally focused on planktonic cells. However, biofilms may be totally different to the 'planktonic' or free-floating bacteria that we have come to understand.[7-9] Indeed, our misunderstanding of the physiology, genetics, physical properties and biochemistry of bacteria found within wound biofilms may result in misguided management such as sequential treatments, low-dose short-term antibiotics and antiseptics, and prolonged treatment with a single biocide.[5,10] Based on this hypothesis, this paper considers the current management strategies that can be used to suppress biofilms.

The planktonic paradigm

Bacteria isolated from chronic wounds are generally cultivated and studied using traditional methods that relate to bacteria in the planktonic state.[5] Once isolated they are concentrated in pure cultures, cultivated in nutrient-rich media, identified and their antibiotic-resistance profiles established.[11-13]

However, planktonic bacteria grown in the laboratory are thought to behave differently to bacteria located on the wound surface.[5,6] This is because microorganisms in the chronic wound bed are considered to exist predominantly within a biofilm community.[8,14,15]

A common medical paradigm for bacteria on the wound surface is termed the 'contamination-infection continuum'.[16] This suggests that individual bacteria land on the wound surface (contamination), find nutrient sources and begin to multiply, replicating outside the host and utilising nutrients on the wound surface (colonisation). Once the individual bacteria have multiplied to reach a critical mass (critical colonisation), they can become recalcitrant to standard clinical therapies.[1] As microorganisms, principally bacteria, within a wound continue to replicate, they begin to invade the host. If the bacteria are able to invade host tissue and are highly virulent, the tissue often becomes infected.

This model projects what we know about the behaviour of single-cell microbes (planktonic) into our view of the wound bioburden. Naturally occurring bacteria attached to surfaces rarely behave like planktonic bacteria.[5] The contamination-infection continuum model, which reflects the planktonic paradigm, needs to be updated to take account of biofilms.[17]

The biofilm paradigm

Biofilms are an ubiquitous problem in industry, dentistry and medicine.[18-20] The National Institutes of Health (NIH) has estimated that up to 80% of human infectious diseases are biofilm related.[21] More than 99% of bacteria found in nature exist in these stable, persistent biofilms, and there are reasons to believe this bacterial theme also holds true in the wound environment.[5,8,14,22,23]

Bacteria encountered in nature and medical diseases are commonly located on a surface, but function in multi-species communities held together by an extracellular slime, known as extracellular polymeric substances (EPS). This slime is composed of polysaccharides, proteins and nucleic acids, and often makes up 80% of the biofilm. The remaining 20% are microbial cells that reside within a microbial community encased within the EPS matrix.[5,10]

The members of the biofilm community possess different genotypic and phenotypic traits, resulting in a structure that is heterogenous, dynamic and recalcitrant to antimicrobials and the immune response.[24] Antibiotics fail to eradicate biofilms due to poor penetration, metabolic inhibition, protected quiescent bacteria (persisters) and other mechanisms. *In vitro* investigations have shown that bacteria in mixed-species biofilm communities can act

synergistically in ways not observed in planktonic bacteria.[25-27] This will, no doubt, change the way clinicians view infection.

Chronic biofilm infections, such as catheter infections, endocarditis and osteomyelitis, often persist indefinitely unless the infected material is removed.[28-30]

This persistence is also evident in chronic wounds.[31] For example, venous leg ulcers can remain open for years, possibly because the host response is unable to clear the biofilm infection. In such cases, it is plausible to suggest the biofilm obtains nutrients not from devitalised tissue, but from plasma and other exudate percolating from the wound bed. The biofilm may even associate closely with blood vessels and so modulate the host's inflammatory response.[32-34] An inadequate blood supply to the infected area — for example, a diabetic foot ulcer — results in a decreased host response, increased biofilm virulence and tissue necrosis.[35] It is possible that the biofilm can manipulate the level of the inflammatory response by modulating its chemical appearance and altering its cell-to-cell signalling activity.[36,37]

Cell-to-cell signalling activity takes place through a quorum-sensing pathway. Quorum-sensing molecules are continuously secreted from each individual bacterium, and act on the same bacterial species, interspecies and even on the cells of their mammalian host. For *Pseudomonas aeruginosa*, acyl-homoserine lactone (AHL) is one of the first discovered and best-known quorum-sensing molecules.

When a critical density of bacteria is present, sufficient quorum-sensing molecules accumulate to upregulate dedicated biofilm pathways and express biofilm phenotype virulence factors, dramatically changing the phenotype of the bacterium.[38] The quorum-sensing pathway can express over 800 new proteins not seen with planktonic phenotype bacteria.[39]

Quorum-sensing inhibitors such as brominated furanones, which occur naturally in the red algae Delisia, can block the receptors for AHL and its isotypes furanones. This holds great promise for the eventual management of medical biofilms.

Incorporating biofilms into the model for microbial infection and wound chronicity may better explain the biochemistry and cellular biology of the chronic wound environment.[14] For example, chronically elevated pro-inflammatory cytokines (tumour necrosis factor-alpha, interleukin-1, alpha and gamma interferons), increased matrix metalloproteases levels (MMP-2, 8 and 9) and increased elastase can be explained by the possible effects of a biofilm on the host's innate immune system.[40] Biofilms may also influence fibroblast senescence, keratinocyte impairment and the failure of endothelial cells to initiate angiogenesis.[40]

Biofilm life

To understand any biofilm infection, it is necessary to understand its life cycle.

A biofilm is initiated when a planktonic bacterium or a fragment of biofilm (cluster of diverse cells embedded in an intercellular matrix) irreversibly attaches to an appropriate surface,[41] such as the exposed extracellular matrix of a wound or an implanted medical device.[42]

Once bound, the bacteria divide and form a microcolony of cells.[41] When a critical density is reached, secreted pheromones (quorum-sensing molecules) and the altered environment within the biofilm cause phenotypic alterations in the bacterial community. The microcolony thus becomes a robust biofilm community that is recalcitrant to the host immune system and to many therapeutic interventions.[39]

Significant alterations occur during biofilm maturation. For example, during the development of a monoculture biofilm, more than 50% of the protein expressed by the bacteria can differ several-fold, depending on the biofilm's stage of development.[39] This enhanced expression of proteins is thought to aid biofilm resistance to antimicrobials and the host's immune response.

The biofilm's strengths are found in its heterogenicity (different protein expression), interspecies cooperation and intercellular matrix structure.[27,43-46]

The most metabolically active cells in the biofilm are located near the non-attached surface where they grow, reproduce, slough and behave similarly to planktonic cells. These metabolically active cells are the most vulnerable to the effects of antibiotics, antiseptics and host defences.

Bacteria that are more deeply embedded in the biofilm's extracellular matrix are sheltered from external perturbations, less metabolically active and more resistant to an array of antimicrobial therapies.[46-48] These protected bacteria can reconstitute the community should a stress destroy the more vulnerable cells at the biofilm surface.[4,8]

It is this ability to remain viable in spite of stresses and to adapt and reconstitute itself that makes the biofilm so tenacious.

The developed biofilm harbours physical and metabolic defences that enable it to resist antimicrobials that typically annihilate planktonic cells.[43,49] These defences include resistance to:

- Ultraviolet light
- Biocides
- Antibiotics
- Host defences.[5,10,27,41,50-52]

Consequently, managing a biofilm community is more difficult than treating planktonic bacteria.

In our opinion, multiple and concurrent strategies may therefore be the most effective way of combating a biofilm infection.[40]

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Biofilm and wounds: theories and practice

We hypothesise that the presence of a biofilm on a chronic wound surface is a barrier to healing. When the skin is broken and a wound forms, the primary host defence to bacterial adhesion and colonisation is compromised.[53] The host defences try to prevent bacteria that seed the wound developing into a chronic infection. However, various host impairments may result in a chronic wound:[54]

- Poor perfusion
- Malnutrition
- Presence of a foreign body
- Pressure
- Repetitive trauma
- Hyperglycaemia
- White blood cell dysfunction.

If a biofilm does become established, its presence in the wound may be difficult to suppress, especially in an individual with a compromised immune system.[5,41] The bacteria and their extracellular components may thus be able to prolong inflammation indefinitely, delaying the normal healing process. It is the presence of a biofilm on the wound surface that, most likely, constitutes its chronic state.[55]

All chronic wounds have bacteria on their surfaces. Despite this, many heal.[56-58] Clinicians often concern themselves with the number (10^5) of culturable bacteria in the wound as this number often correlates to the amount of immune stimulation (ie, classic signs of acute infection) seen in the patient.[56]

However, a clinical biofilm bacteria may not culture, but can still be viable.[43]

In addition, it is thought that biofilm bacteria do not initiate the potent inflammation that groups of planktonic, highly virulent, bacteria may produce. Biofilms, therefore, can be overlooked using traditional sampling techniques.[5,41,59]

Biofilm management strategies

Physical

In our opinion, physical intervention is vital to the successful management of biofilms.[31] Sharp debridement significantly reduces the physical presence of microorganisms and devitalised host components.[60] We believe that debridement not only removes bacteria, but also exposes host defences that are more intact and better suited to combat bacteria. It can be used to remove biofilm,[61] although the biofilm's ability to reconstitute itself makes debridement alone insufficient.

As a result, topical antimicrobial and antibiofilm agents should also be considered.[60] This approach, coupled with appropriate wound dressings, will reduce the number of planktonic bacteria that have become dispersed from the biofilm and prevent re-attachment of bacteria to the wound.

This will in turn prevent potential regeneration of the biofilm.

Wound dressings

Many wound dressings are not inherently antimicrobial, but are known to help reduce bacterial load and acute infection rates.[62,63] Wound dressings that do not foster biofilm growth on their surface may be important,[24,64] although this is a poorly researched area and its significance is open to debate. In addition, contact between the dressing and wound bed prevents pockets of open spaces at the wound surface, which are prone to microbial colonisation and, in turn, biofilm development.[24,64] Some wound dressings may have 'antibiofilm' qualities, based on their physical and chemical attributes alone.

Antimicrobials

- **Antibiotics** Antimicrobial agents, specifically antibiotics, are thought to suppress the biofilm's metabolically active cells, which are the most detrimental to the host tissue because of their ability to upregulate host inflammation.[55] However, a large portion of the biofilm is composed of dormant cells that do not respond well to antibiotics.[10]

Systemic antibiotics are warranted when there is significant wound infection involving deep tissues, such as in diabetic foot ulcers, or when clinical findings or laboratory markers suggest the infection is systemic.[65] Despite this, systemic antibiotics have been documented to be only 25–32% effective against biofilms,[66,67] resulting in only transient suppression of the biofilm at its outermost active edges. The clinical signs of infection often recur after the antibiotic regimen is complete because the antibiotics only suppress rapidly growing cells.[68] Consequently, the recalcitrant biofilm remains and the suppressed cells begin to metabolise rapidly.[43] This recalcitrance of biofilms to antibiotics is not evident in *in vitro* planktonic testing.

Treating biofilm infections with antibiotics is even more problematic with ischaemic wounds, when appropriate levels of antibiotic may not reach the infection.

Despite the limited efficacy of systemic or topical antibiotics, they are thought to contribute significantly to the clinical management of wound biofilms.[46] However, as no single strategy has proved consistently effective in suppressing an entire biofilm,[31] we believe that antimicrobial are most useful when combined with other strategies, such as debridement.

- **Antiseptics** Once the biofilm has been adequately managed with debridement and systemic antibiotics, topical antiseptics can be considered as they further suppress the biofilm community. Antiseptics can penetrate biofilms and cause significant microbial death. However, some have been shown to damage human proteins, such as antibodies and cytokines, and to kill human cells.[69]

Clinicians have thus suggested they be used selectively and sparingly in chronic wounds.[70]

- **Ionic silver** Silver can exert bactericidal effects at minute concentrations.[71] Ionic silver is efficacious against a broad range of microorganisms,[72,73] and some silver dressings have the potential to prevent biofilms *in vitro*.[24] Laboratory studies have compared silver dressings from different manufacturers,[74,75] with some positive results on biofilms.[24,76] However, a recent *in vitro* study concluded that 'the concentration of silver in currently available wound dressings is much too low for treatment of chronic biofilm wounds'.[77] This study was conducted using only *in vitro P. aeruginosa* biofilms and not *in vivo* polymicrobial chronic wounds. In contrast, Chaw[78] showed that ionic silver levels as low as 50ppb help to destabilise the matrix of *Staphylococcus epidermidis* biofilms.

Despite a preliminary understanding of the genetics underlying silver resistance[79-81] and some available evidence on prevalence,[82,83] resistance is not considered a problem, given the positive clinical outcomes.

- **Iodine** This is used to prevent wound infection and aid healing. However, there is debate not only on its antimicrobial efficacy and chemical stability, but also on its toxicity to host tissues and the enusing effect on patient comfort.[69,84]

Povidone-iodine is not as effective as some other biocides in eradicating *S. epidermis* within *in vitro* biofilms,[85] but cadexomer iodine provides enough iodine for biofilm suppression while not causing significant host damage.[86]

- **Honey** It has been claimed that medicinal honey has antibacterial activity and can promote healing.[87,88] *In vitro* studies with cell lines exposed to honey solutions have demonstrated that it modulates monocytic cell activity. It has been speculated that this influences the wound healing process,[89,90] although this has not been fully explained. It has been suggested that the osmotic potential of honey is its key mechanism of action, which may have effects on biofilms, but others suggest that its phytochemicals may be more important.

Antibiofilm strategies

Varous antibiofilm agents are available. Some have been used in wound care and others are being investigated *in vitro*. Well-known antibiofilm agents include:

- Lactoferrin[91]
- Ethylenediaminetetraacetic acid (EDTA)[92-94]
- RNA III inhibitory peptide[95]
- Dispersin B[40]
- Gallium[96]
- Acetyl salicylic acid[97]
- Many other plant-derived agents.

These agents interfere with cellular communication, disrupt the biofilm's intercellular matrix or alter cell metabolism. They do this without impairing the growth, reproduction or integrity of the microbial cells.[41,91,96,98] Lactoferrin, for example, does not appear to harm the bacteria but instead blocks their adherence to a surface, thus preventing the first necessary step in biofilm formation.[91]

As antibiofilm agents are not necessarily toxic to any cells (human or bacterial), some may play a potential role in wound care, where the clinician needs to suppress the biofilm without destroying the host cells.

Lactoferrin

Bovine lactoferrin is a protein that has been used to protect exposed meat surfaces from bacterial biofilm formation. It can block the attachment of planktonic bacteria to a surface,[91] thus stopping the first step in biofilm formation.

Initial work on its antimicrobial activity suggested that the protein's affinity for iron (transferrin) was its main mechanism of action.[99] Lactoferrin sequesters iron, depleting this essential bacterial nutrient and so causing a bacteriostatic action.[100] However, this and subsequent studies focused on planktonic bacteria, not biofilms.[101,102]

Lactoferrin has been found to have a direct bacteriocidal effect on planktonic bacteria,[101] by binding to the lipopolysaccharide portion in the outer membrane of Gram-negative bacteria. This causes the rapid release of lipopolysaccharides, which increases membrane permeability and so causes planktonic bacterial cell death.[101]

Lactoferrin works synergistically with polymorphonuclear cells to produce bacteriocidal activity.[102] It acts as a reservoir for iron, which is required to catalyse hydroxyl radical production, one of the main weapons in the polymorphonuclear cell's armamentarium.

Lactoferrin remains stable and continues to bind iron even at very low pH. The activated neutrophil binds lactoferrin-containing granules within the acidic phagolysosome, resulting in the needed iron source for its bacteriocidal activity.[102]

Interestingly, the very first property identified for lactoferrin, its transferrin activity (which contributed to its name), is re-emerging as one of its most significant contributions to host defences. Lactoferrin's iron-binding capacity is an important inhibitor of biofilms.

Xylitol

Xylitol, a five-carbon alcohol sugar, is a naturally occurring substance commonly used in chewing gum, and can reduce the incidence of dental carries. It has been suggested that it interferes with biofilm formation.[103]

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Katsuyama et al.[104] showed that use of xylitol along with farnesol synergistically inhibited biofilm formation in patients with atopic dermatitis. In this laboratory study, the microflora of healthy humans and patients with atopic dermatitis that cultured *S. aureus* showed inhibited biofilm formation.

In their study using scanning electron microscopy and evaluating the results, Masako et al. found xylitol inhibited the formation of glycocalyces through unknown pathways.[105]

Gallium

Gallium has an ionic radius is very similar to that of iron (Fe). Many biologic systems, therefore, are unable to distinguish Ga3+ from Fe3+. Gallium can disrupt Fe-dependent processes that are essential for bacterial growth and proliferation.[106]

Galium is already approved by the FDA in the US, and large doses are given (intravenously) to treat hypercalcaemia.[107]

Low doses of gallium nitrate have been shown to interfere with biofilm development.[99]

The transition metal gallium disrupts *P. aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity.[96]

EDTA

Ethylenediaminetetraacetic acid (EDTA) is widely used as a metal-chelating agent in the food and water industry, and for medical symptoms such as lead and heavy metal poisoning.[108]

Investigations into the effect of EDTA on bacteria started over 40 years ago.[109]

Disodium EDTA has been shown to have a bactericidal effect against clinical isolates of *S. epidermis* and to prevent attachment of bacteria to catheter segments *in vitro*.[110]

Research on catheter biofilms, using the tetrasodium salt of EDTA, showed that 40mg/ml tetrasodium EDTA had a broad spectrum of activity against *in vivo*-generated biofilms, showing effective biofilm removal after 24 hours' incubation using viable counts and scanning electron microscopy (SEM).[92,93]

Recently, Martineau and Dosch[111] showed that sodium EDTA incorporated into a wound gel enhanced its antibiofilm properties on *P. aeruginosa* biofilms.

Dispersin B

Dispersin B is a bacterial enzyme that has been isolated and exploited for antibiofilm usage. It has principally been investigated in dentistry, and various studies have shown that it inhibits biofilm formation or causes biofilm detachment.[112-115]

The principal target of dispersin B is the EPS of some types of biofilm, and it works by degrading the community structure of the biofilm.[116]

Other antibiofilm management strategies

Bacteriophages

Bacteriophages (a virus that affects bacteria) have been shown to be effective in managing infection, mainly because of their bactericidal activity.

Bacteriophages only replicate at the site of infection, and will acculumate in areas in which their target bacteria reside.[117]

To date, there have been no serious side-effects due to phage therapy, despite its use on experimental infections such as meningitis and septicaemia.[118]

Studies involving the interaction of bacteriophages and biofilms have shown that phages can degrade biofilm exopolysaccharide and infect biofilm cells,[119] when experimentally tracing the interaction of bacteriophage with bacterial biofilms using fluorescent and chromogenic probes.[120]

Glucose oxidase

Used in combination with other enzymes, glucose oxidase has been shown to have effects on biofilms.[121] For example, a mixture of polysaccharide-hydrolysing enzymes removed bacterial biofilm but did not seem to have significant bactericidal activity. The authors concluded that, by combining oxidoreductases with polysaccharide-hydrolysing enzymes, bactericidal activity can be enhanced and biofilms removed from surfaces.[121]

Pulsed electric fields

Pulsed electric fields (electrical stimulation at a low voltage -0.5 to 5v) have been shown to prevent *P. aeruginosa* biofilm development.[122]

Overall management strategies for biofilms

We suggest that concurrent (simultaneous) management strategies are more likely to suppress a biofilm infection for a prolonged period, than single or sequential treatment strategies.[98,112,120,123]

In practice, this means that when a biofilm is suspected of delaying wound healing, the most appropriate dressing should be used first. This will, of course, depend on the exudate level, but it should also aim to remove and absorb the detached sections of the biofilm and circulating planktonic cells. The dressing would need to contain an appropriate active agent.

An 'antibiofilm' strategy should then be employed. The aim is to help suppress the biofilm, reduce the bacterial load and aid the immune response. We propose that extensive biofilm suppression may be essential to allow the impaired host to promote healing.[41] Because of the biofilm's diverse defences, it is essential to use a strategy that suppresses the biofilm while simultaneously easing the host's burden by facilitating wound healing.

Two general types of antimicrobial agents could

be used to help suppress and eradicate biofilms.

- The first antimicrobial targets the microbial cells' metabolism or integrity. These are traditional systemic antibiotics and topical antiseptics
- The second type of antimicrobial are those referred to as 'antibiofilm agents' above. While rarely used in practice at present, they will probably become a vital addition to the clinician's wound management toolbox in the coming years.

Conclusion

In our opinion, it is reasonable to conclude that microbial organisation on the wound surface is a biofilm phenotype. This microbial community presents multiple obstacles to the clinician when attempting to heal a chronic wound. Biofilms are considered to be resistant to many biocides, antibiotics and wound-care products. However, strategies are available that can be used to suppress biofilms until better tools are developed and made commercially available.

The mainstay of managing biofilm is its frequent removal from the wound surface, either with sharp or surgical debridement. At present, the most effective treatment of medical biofilms is its physical removal.

The early biofilm that re-emerges after debridement needs to be suppressed with multiple antibiofilm strategies. These may include the use of non-toxic wound cleansers, topical antimicrobials and advanced primary dressings, which work together to suppress the biofilm.

Since biofilms can adapt to selective stresses, some clinicians have found a rotating regimen of selective antiseptics, such as silver or iodine, advantageous. (By rotating, we mean changing to a new biocide for an appropriate period time — for example, every four weeks.)

Furthermore, multiple antibiofilm agents, which target different colony defences, may be used concurrently. This will make topical antiseptics and systemic antibiotics more effective. Concurrent systemic antibiotics may further suppress the biofilm.

As well as selectively targeting the biofilm in the wound, it is appropriate to address other barriers to healing that are complicating the disease, such as glucose levels, oedema, repetitive trauma and vascular integrity. Addressing these complications will help augment the host's defences which, when working optimally, provide the best means of wound management.

A well-designed, protocol-driven regimen will be needed to organise the simultaneous use of all these strategies. However, concomitant strategies are needed to defeat such a determined enemy as a

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