Surgical site infections: biofilms, dehiscence and delayed healing

Biofilms are slowly becoming recognised as a cause of wound infection. Typically, the biofilm delays healing without inducing a dramatic host response. Biofilms may be unperturbed by antimicrobial or neutrophil attack and can survive in a relatively harsh environment, resisting attempts at removal. Their presence is often associated with chronic wounds but they can also be involved in acute wound infection. Acknowledging their presence as a potential cause of surgical site infection may explain the sometimes disappointing response obtained from traditional approaches such as promoting drainage, systemic antibiotics or delayed closure.

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Although research into bacterial biofilms has been extensive in industry and dentistry over the past two decades it has only been within the past 10 years that they have been investigated in relation to modern medicine. Given the documented association between biofilms and infections and the incurring cost this inflicts on healthcare systems, there is certainly a need for a greater understanding of biofilms in this area.

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Biofilm formation

Bacteria are known to exist as a biofilm phenotype within the natural environment (Callow and Callow, 2006). These biofilm micro-organisms exist in an organised communal microbial ecosystem. These dynamic ecosystems are found attached to surfaces and encased within an extracellular matrix of polymeric substances (Costerton et al, 1995; Potera, 1998). Within the microbial biofilm community, the residing micro-organisms are known to be quite different, both phenotypically and genetically, from their free-floating 'planktonic' counterparts. Historically, planktonic micro-organisms — most specifically, bacteria — are those which have been commonly studied during standard laboratory research and antibiotic sensitivity testing. Very distinctive physiological and biochemical differences have been observed between bacteria in the planktonic and the sessile state.

Bacteria residing within biofilms are known to be regulated by diffusible molecules or 'pheromones' which aid in the expression of proteins of individual bacterium providing them with enhanced survival strategies (Stoodley et al, 2002). These communication molecules are called quorum-sensing molecules, and they regulate a number of pathways within the bacterium, causing up to 800 new proteins to be expressed (Sauer et al, 2002). The change in a single bacterium from the planktonic state to a sessile community-based phenotype, is considered as radical as the metamorphosis of a caterpillar into a butterfly, as both are genetically identical yet phenotypically quite different.

Such differences may provide insight as to why the link between biofilms and delayed wound healing and infection control is significant and of paramount importance to clinical outcome.

Defence mechanisms

An understanding of biofilms, in particular their defense mechanisms, is fundamentally important to help guide wound management strategies. Research has shown that biofilms may be totally unperturbed by activated macrophages, neutrophils, antibodies, complement, or other host defenses (Leid et al, 2002; Fux et al, 2005). Within the host, the biofilm is able to highjack many of the host's components such as fibrinogen (Masako et al, 2005), neutrophil DNA (Walker et al, 2005) and collagen to incorporate into its protective matrix, making it impervious and impregnable to host attacks (Leid et

al, 2002). Biofilms are also recalcitrant to biocides, drying, overhydration, or other environmental stresses (Fux et al, 2002).

The up-regulation of pro-inflammatory cytokine production characteristically found in chronic wounds may be explained by the presence of biofilm infection leading to the production of exudate from surrounding capillaries. This highly nutritious exudate will percolate through the biofilm, and provide nutrients to the resident microbiota of the biofilm. The supply of 'food' helps to maintain biofilm security and the sustainability, stability and fitness of the biofilm. Consequently from an evolutionary perspective a highly virulent biofilm will have a selective advantage based on its microbial composition and net pathogenic effect and therefore enhanced survival rates when compared with a less virulent biofilm.

The differences between planktonic and biofilm phenotype bacteria become pronounced when observing their interaction with the host. Planktonic bacteria are very competitive during the development of an ecosystem (Lu et al, 2002). This can be shown by placing two planktonic bacteria on an agar plate with the appropriate nutrients. Research has shown that the two planktonic bacteria will compete (Cain et al, 2000; 2003). The dominant or more virulent species of bacteria will out-compete the other bacteria in close proximity and will overgrow on the agar plate. This is similar to what may occur in planktonic infections in humans.

After a bacterium attaches to any surface it secretes a matrix material composed of polymeric sugars, proteins and/or DNA. This matrix material helps the bacterium to secure itself to the surface and helps to protect the colonising microbiota from environmental and host stresses. As the bacterium begins to grow and multiply it forms an aggregate of cells called a microcolony. As the bacteria within the microcolony continue to divide, a critical density of bacteria or quorum develops that allows the microcolony to develop further. As these microcolonies progress further they 'climax' microbiologically to form a mature polymicrobial biofilm (Stoodley et al, 2002). The hallmarks of a mature biofilm is the formation of a three-dimensional structure with an organised arrangement of water channels or capillaries which run deep into both the biofilm and the host, an architecture considered akin to a multicellular organism. Such a structure is able to adapt to outside perturbations while maintaining a 'quasi' state of homeostasis.

Biofilms and surgical site infections

There is a high probability that micro-organisms will contaminate the site of incision or excision in a surgical wound. If a bacterium is able to attach onto a biological surface it rapidly changes the proteins it expresses and as such becomes sessile and significantly different phenotypically, when compared with its planktonic counterpart (Costerton et al, 1999).

Surgical site infections (SSIs) are those that develop within 30 days after an operation or within one year if an implant was placed and the infection appears to be related to the surgery. Postoperative SSIs are a significant cause of postoperative morbidity and account for 2–14% of all surgical complications (Graves et al, 2006). In the USA, between 500,000 and 750,000 SSIs occur annually (Perencevich et al, 2006; Edmiston et al, 2006). Most SSIs, which occur as a result of contamination from exogenous sources and the patient's own indigenous microbiota, take between 5–10 days to manifest after a surgical procedure (Leaper and Snyder, 2008). Such wound infections have a major impact on surgical performance and contribute extensively to an increased burden to healthcare costs. More important is the fact that a wound infection increases a patient's morbidity. Consequently, this extends patient distress which in turn, further hinders wound healing. Additional complications to wound healing include postoperative dehiscence — a possible result of a 'sub-clinical' or biofilm infection. Such a complication presents a management challenge to clinicians, as the wound does not always heal promptly and does not respond to traditional approaches such as promoting drainage, systemic antibiotics or delayed closure.

Acute wound infections tend to be progressive with a significant host response illustrated by the classic signs of Celsus, including erythema, swelling, heat and pain. Their natural history is one of rapid manifestation, prompt tissue destruction and then resolution. These infections are susceptible to systemic antibiotics and quickly resolve within a 10–14-day course of appropriate treatment (Leibovitz, 2003). Planktonic phenotype bacteria explain much of this behaviour. Planktonic bacteria up-regulate virulence factors, bacterial proteases, and other secreted agents to lyse tissues on which it feeds (Overhage et al, 2008). The percieved pattern of acute planktonic infection is one of predation: if the host does not adequately respond or if there is not an outside intervention, the host will die.

In contrast, infection caused by the biofilm phenotype bacteria is significantly different to planktonic-related bacterial infections. Once the biofilm is established on the surface of the host, regardless of the environment (e.g sinus, gut or skin), the sessile bacteria exhibit significantly different strategies known to enhance their survival within the inherent biofilm community.

The differences often observed between acute and chronic infections could be best explained by the presence of a pathogenic or bad biofilm. Chronic infections follow a persistent undulating course with frequent exacerbations (Costerton et al, 1999) and will generally respond incompletely to systemic antibiotics, often reemerging once the systemic antibiotics are withdrawn (Fux et al, 2005). A hallmark of

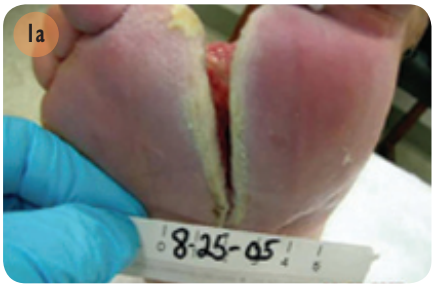


Figure 1a. A surgical wound on the plantar region of the foot one week after sutures were removed. the surface has been aggressively debrided an all undermined tissue opened at the distal end.

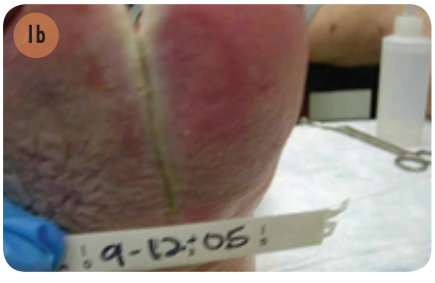


Figure 1b. The wound two weeks later — the plantar area is healed.

many, if not most, chronic infections is that they will respond marginally to systemic antibiotics as well as immunosuppressants such as steroids but the response will be short-lived and will not be sustained (Mandel et al, 1999). The response of chronic infections to steroids is possible indirect evidence that the inflammation stimulates the production of plasma exudate in the area of infection which is necessary for the biofilm to thrive. Biofilms may help to explain much of the observed atypical behaviour seen with some surgical site infections that lack the traditional host response seen in acute infections.

Many surgical site infections occur after a patient has been discharged from hospital and such infections develop slowly, which is very different to acute infections. The incision may dehisce in part or in whole and often there is no damage to tissue surrounding the wound, with degradation confined to the surface of the surgical incision. Biofilms are more successful on surfaces — especially surfaces that are in contact with another such as sutures, wound dressings and wound tissue (Otto, 2008). The biofilm phenotype on the surface of the surgical wound may help to explain why the strategies we use to heal wounds caused by planktonic bacteria fail to prevent the wound from dehiscing.

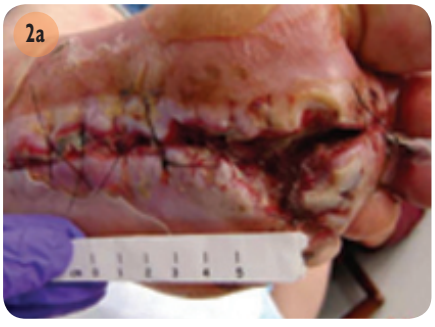
Biofilm management for surgical site infections is based on multiple concurrent strategies specifically targeting biofilm behaviour (Wolcott and Rhoads, 2008). It includes clearing the wound of any tunnelling or undermined tissue by removing sutures or opening skin to expose the surface-associated bacteria. This robs the biofilm of a second surface to organise around and it also allows access for adjunct strategies such as regular debridement. The biofilm can be deprived of its nutritional source by immunosuppressants, but this also blocks the host's healing responses and should be considered a last resort. It could be hypothesised that the rapid removal of exudate from the wound bed through use of negative pressure wound therapy or super-absorbent dressings may accelerate the transit of exudate through the biofilm, thus preventing full extraction of nutrients and their utilisation by the biofilm.

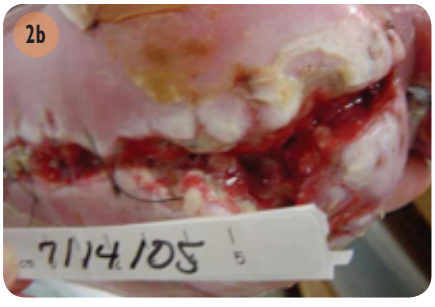
Frequent debridement of the surface of the wound forces constant reconstitution of the biofilm, making it more susceptible to topical and systemic antibiotics and selective biocides (Stewart, 2002; Stewart et al, 2001). Prescribing high doses of systemic antibiotics for extended periods of time, as may occur in the management of bacterial endocarditis and osteomyelitis, may suppress the biofilm (Fux et al, 2005). As a sole strategy, topical and systemic antibiotics are unable to successfully manage biofilm phenotype bacteria and should only be used in conjunction with other tactics. In vitro evidence suggests some positive outcomes for biofilm management based on the bacteriocidal activity of ionic silver (Percival et al, 2008; Chaw et al, 2005) and honey (Okhiria et al, 2006). Personal empirical evidence also indicates that certain iodine products are effective at suppressing biofilm phenotype bacteria without harming the host's defenses. Consequently it is feasible to propose that multiple concurrent strategies that specifically target biofilm phenotype bacteria, may be beneficial in aiding successful wound healing by eradicating surgical site infections.

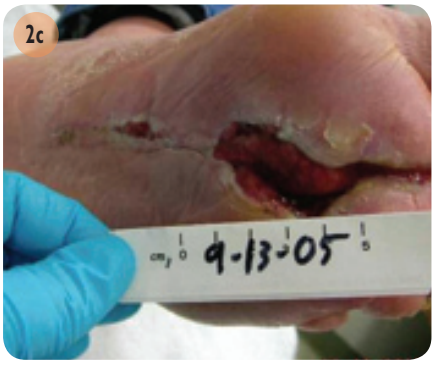
Recent clinical evidence (Wolcott and Ehrlich, 2008) has shown that biofilms are best managed through physical disruption. This approach has been proven in drinking water pipes, whirlpools, hot-tubs, toilets, and on our teeth. It has also been demonstrated in food packaging, food processing and swimming pool maintenance. By frequently disrupting the biofilm with brushes or by other physical means, microcolonies will be significantly degraded. This principle of physical removal of biofilm with a temporary increase in vulnerability can be exploited in surgical site infections in several ways. Essentially by promptly laying open the involved area of the wound and regularly removing any dead and devitalised tissue, physically managing the surface of the wound will help to suppress the reaccumulation of biofilm. In the authors' opinion debridement should be employed at least weekly for biofilm-related infections. However, as efficacious as physical disruption of a biofilm is, it is rarely sufficient for total biofilm suppression. By adding other simultaneous strategies such as topical biocides, anti-biofilm agents and systemic antibiotics, it may be possible to further suppress the re-accumulation and resuscitation of the biofilm.

Case reports

Several cases involving surgical site infections treated by biofilm-based wound management in an outpatient wound care center setting were prepared. The following four case studies of different management strategies for surgical site infections illustrate some important clinical points. *Figure 1* illustrates a







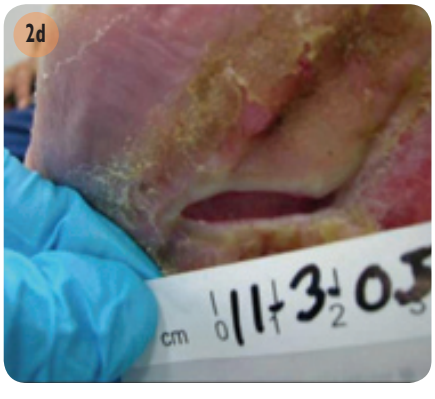
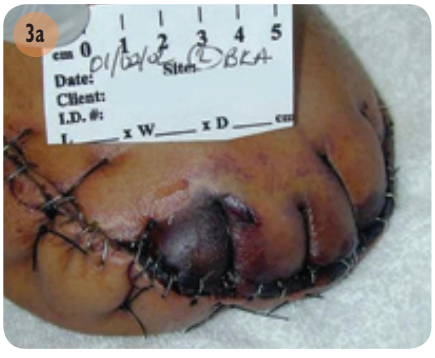


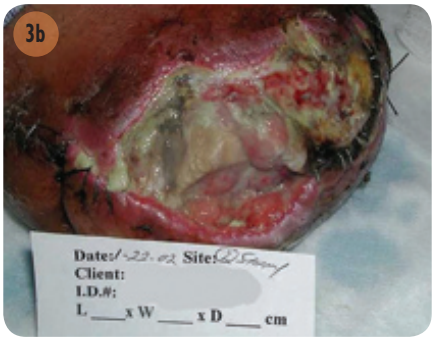
Figure 2a. The initial visit by a patient with diabetes with severe peripheral neuropathy two weeks after an elective surgery for a Charcot foot.

Figure 2b. The retention sutures were removed and the wound bed exposed to prevent wound surfaces from touching and for frequent debridement.

Figure 2c. Frequent management of wound biofilm produced consistent healing.

Figure 2d. Continued off-loading and management of other comorbidities along with continued suppression of wound biofilm precluded complete wound healing.





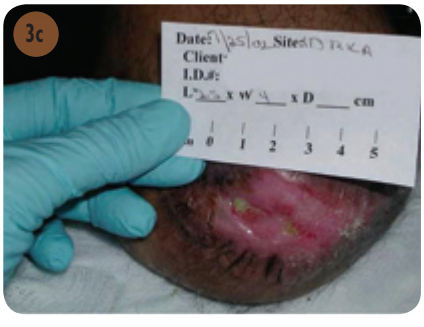
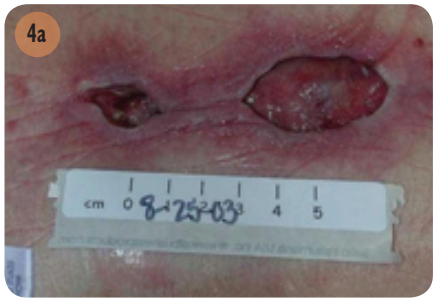
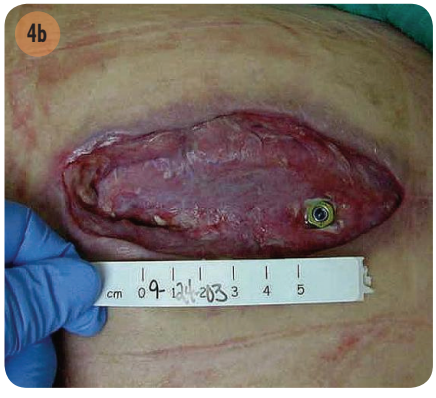


Figure 3a. This below-knee stump of an immunosuppressed patient secondary to renal transplant and diabetes shows significant distress just one week post-surgery.

Figure 3b. Three weeks later the wound shows complete dehiscence with exposure of the tibia and a culture of methicillin-resistant *Staphylococcus aureus*.

Figure 3c. Even though bone was exposed and the patient had MRSA contributing to the wound biofilm, the principles of altering the anatomy of the wound to remove surfaces that touch, frequent removal of wound biofilm and strategies to prevent wound biofilm reaccumulation resulted in complete healing over the exposed bone.





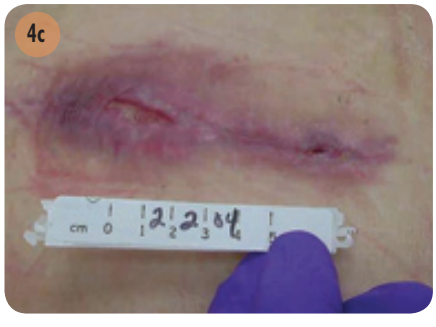


Figure 4a. The patient presented three weeks post-op for instrumentation of her thoracic spine. There was undermining between the two areas of dehiscence with exposed hardware.

Figure 4b. The area of tunneling between the two wounds was opened and the undermining at the edge of the wound was removed. Wound biofilm was managed on a weekly basis with strategies to prevent reaccumulation.

Figure 4c. Fourteen weeks later the patient demonstrates complete healing over the exposed hardware.

counterintuitive point that the more aggressively the wound is opened the quicker it heals. *Figure 1a* shows the wound one week after sutures have been removed and the surface of the surgical wound aggressively debrided and all undermined tissue opened at the distal end. A little over two weeks later the entire plantar portion of the wound was healed (*Figure 1b*). This demonstrates that when a biofilm is adequately suppressed, the wound heals at about the same rate as would be expected in an acute wound (a wound without a biofilm) even in patients with

diabetes who normally have a delayed response to healing.

Figures 2a–d illustrates a plantar ulcer in a patient with diabetes and suggests that trying to keep the wound artificially 'pulled together' by leaving some of the sutures in situ is unnecessary (Volfson et al, 2008). Opening the wound and removing contiguous surfaces produces two beneficial effects. First the biofilm is more easily suppressed and second the wound can contract at very high rates (Wolcott and Rhoads, 2008).

The dangers of trying to hold the wound together when the biofilm has established itself are depicted in *Figure 3*. In this example the patient presented with a painful stump which was a poor colour, necrotic and draining from many areas along the wound margin. By leaving staples in place, the time to complete healing was markedly delayed. It should be noted that the patient had exposed bone in the base of the wound with the distal end being very soft, consistent with the presence of osteomyelitis. The patient was able to heal over the exposed, infected bone and become a functional prosthetic wearer, allowing tissue to grow over the bone. By following a biofilm-based wound strategy where the wound was opened and the biofilm was frequently removed from the surface followed by using selective biocides, such as silver, cadexomer and iodine to prevent the reaccumulation of biofilm in conjunction with antibiotics. When multiple strategies are used simultaneously this is the more effective way to suppress biofilm. Aggressive, early removal of sutures/staples, opening, undermining and removing devitalised tissue was shown to improve the time the wound took to heal.

The ability for a wound to successfully heal when tissue surrounding implanted medical devices have become infected may be seen in *Figures 4a–c*. The patient was an elderly woman who had a surgical intervention for spinal cancer. Standard treatment for infections of implanted medical device in this situation would be removal of the device. In this instance a biofilm-based strategy was implemented including the use of selective biocides, anti biofilm agents (such as actoferrin and xylitol), appropriate antibiotics and frequent debridement, which avoided removal of the medical device and yet encouraged wound closure. Eighteen months later, the wound remained closed.

Conclusion

It has been recognised for many decades that bacteria can live in surface-associated communities, called a biofilm (Costerton et al, 1978). It is now well established that biofilms are implicated in certain diseases and infections (Del Pozo and Patel, 2007). It is only recently, however, that the presence of biofilms in chronic wounds has been clinically recognised (James et al, 2008) despite many years of speculation (Harrison-Balestra et al, 2003; Percival and Bowler, 2004). The role biofilms may play in chronic infections and delayed healing is presently under investigation (Wolcott and Ehrlich, 2008). Surgical site infections are considered to have many of the characteristics that have been observed in chronic wounds specifically and chronic infections in general. A better understanding of biofilm phenotype bacteria will help in the understanding and management of surgical site infections (Soderquist, 2007).

The expression of new proteins that occurs in bacteria as they change into the biofilm state, along with the generation of a protective matrix aids in the recalcitrant attachment of a microbial community to the host's incredible protective armoury. Despite the host's defenses such as proteases, white blood cells (Leid et al, 2005), antibodies (Lam et al, 1987), and other immunological responses (Kristian et al, 2008) their efficacy is somewhat limited and remain only minimally effective against the biofilm. Efforts to strengthen the host such as normalising blood sugar, correcting anaemia, or managing other systemic diseases, will only make small incremental improvements in wound healing. Acute surgical wounds are generally able to counter the threat of infection when challenged by planktonic bacteria. A weakness in the host's immune system becomes apparent when the threat is posed by (biofilm) bacteria that are able to attach to the wound surface and evade the normal host response to invasion by alien cells. One possible approach to managing biofilm infection is to find a way of bolstering the host's immune defences to this form of bacterial onslaught.

Through suppression of the biofilm, host healing processes like angiogenesis, the formation of an extracellular matrix, and wound contraction becomes much more effective. It has been demonstrated that by targeting the biofilm, a higher percentage of chronic wounds heal showing that the biofilm is an important barrier to healing (Wolcott and Rhoads, 2008). This information suggests that early intervention with aggressive, multiple concurrent strategies targeting the surface-associated bacteria on the surgical site infection may result in improved outcomes for these wounds. WUK

Key Points

- Biofilms are a significant cause of delayed healing in wounds.
- Biofilms are resistant to traditional approaches of managing wound infection.
- Biofilms are a potential cause for dehiscence and delayed healing in surgical wounds
- Biofilm-based wound care is a management approach based on multiple concurrent strategies specifically targeting biofilm behaviour.

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