Biofilms: the myths and realities

There has been an increasing interest in biofilms and their possible contribution to the non-healing of chronic wounds. Currently, there is no gold standard for identifying if a biofilm is present, and many strategies are still under investigation to establish the best way to treat wounds that may have a biofilm present. This article seeks to address some myths surrounding biofilms and discusses current recommendations that may assist clinicians to treat wounds with a possible biofilm in clinical practice.

"There still remain many unanswered questions on how biofilms affect wounds and what is the best way to treat wounds which have been colonised by a biofilm in order to improve healing rates."

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Biofilms are free floating bacteria that have encased themselves in a thick polymeric substance made up of sugar and proteins. The encased bacteria attach themselves firmly to a surface forming biofilm colonies that are able to protect themselves from many external threats making removal or eradication difficult (Phillips et al, 2010)

Biofilms are not a new phenomenon and have been shown to cause problems in areas such as natural aquatic environments and industrial aquatic environments (Costerton et al, 1987). Although biofilms were identified over 40 years ago, there has been much sceptism around a biofilms ability to cause harm, thus the possible issues have been slow to be addressed. This is may be due to the absence of robust research available, poor understanding of the effect that a biofilm may have and lack of routine method of sampling to detect biofilm species (Gottrup et al, 2013).

Within healthcare, the development of biofilm colonies have been identified on medical devices, such as catheters, endotracheal tubes and intravenous lines (Stickler, 2008; Vertes et al, 2012). Within the body, biofilms have been cultured on surfaces such as skin, respiratory tract, epithelium, skin and gut (Thomson, 2010). Biofilms are thought to cause chronic infection and contribute to prolonged inflammation, they are difficult to treat due to resistance to antibiotics and protection from the bodies natural defenses. In chronic diseases such as cystic fibrosis patients are already susceptible to infection, formation of biofilms in these patients may lead to unresolved infection and contributes to disease progression (Bjarnsholt et al, 2009).

Within wound care, there is an increasing interest in the effects of biofilms on wound healing (Thomson, 2010). James et al (2008) indicated that up to 60% of chronic wounds might have a biofilm present and it is believed that biofilms may impede wound healing (Gottrup et al, 2013). However, there still remain many unanswered questions on how biofilms affect wounds and what is

the best way to treat wounds that have been colonised by a biofilm in order to improve healing rates (Thomson, 2010). This article will attempt to address some of the myths surrounding biofilms and will present current recommendations for treating a wound that may be them.

What is a biofilm? Do they exist?

Yes, they do exist. Microorganisms, such as bacteria, are very adept at adapting to survive and protect themselves from eradication. Bacteria start out as being planktonic in nature — free-floating and unattached from the wound bed. At this point, systemic antibiotics or topical antimicrobials can inhibit or kill planktonic bacteria and are considered an effective means of eradicating bacteria present in a wound (Keast et al, 2014). Biofilms are free-floating bacteria that have encased themselves in a thick polymeric substance made up of sugar and proteins known as extracellular polysaccharide matrix. The encased bacteria attach themselves firmly to the wound surface forming biofilm colonies. This attachment is at first reversible (Phillips et al, 2010). The attached biofilm starts to multiply and the matrix protects the biofilm from damage and is impermeable to antibiotics, antimicrobials and the hosts' immune response. At this stage, the biofilm attachment becomes irreversible. Currently, debridement may be the only means identified as being effective in disrupting a mature biofilm (Gottrup et al, 2013).

Biofilms can consist of a single species of bacteria or fungi or can be polymicrobial, in which several species of bacteria may be present. A study by Thomsen et al (2010) revealed that chronic wounds may contain an average of 5.4 species of bacteria within the wound bed. Bacteria within the biofilm start to communicate with each other by a process called quorum sensing. This process enables the bacteria within the biofim to respond to any changes in the environment, which further assists in its survival (Phillips et al, 2010).

Planktonic bacteria are now continuously dispersed from the matured biofilm and are able to attach themselves to other parts of the wound to form new biofilm colonies (*Figure 1*). It has been demonstrated that a mature biofilm can form *in vitro* within 2–4 days (Costerton, 1984).

Table 1. Risk factors that may prevent healing and increase a patient's risk of developing an infection or a biofilm forming at the wound site.

Patient-related factors	Wound-related factors
Immunosuppression	Chronicity of the wound
Malnutrition	Size of the wound
Diabetes	Previous infection
Decreased tissue perfusion	Site of wound
Oedema	Excessive moisture in the wound
Smoking	

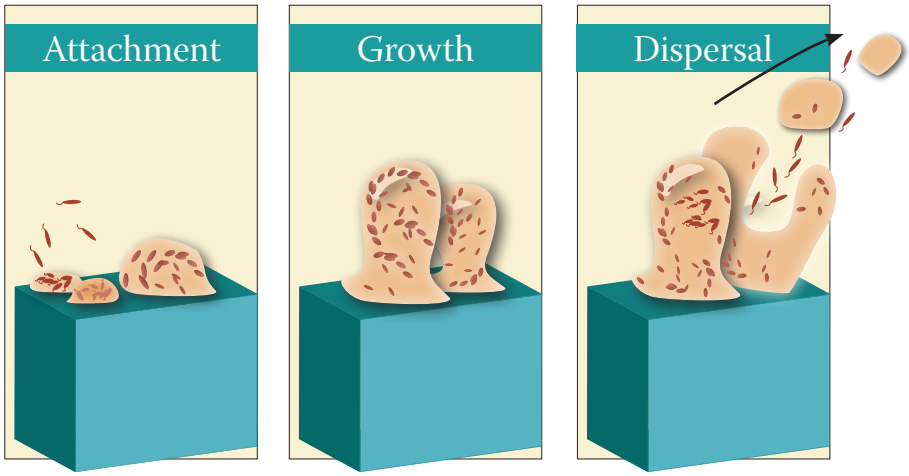

Figure 1. The biofilm life cycle: attachment, growth of colonies (growth) and periodic detachment of planktonic cells (dispersal).

Are biofilms detrimental to wound healing?

Yes. Biofilm colonies have been detected in wounds that have failed to heal (Davis et al, 2008; James et al, 2008). There may be a link between biofilms and chronic inflammation, however, the exact influence of biofilms and their mode of action is not fully understood. Further research is required to establish exactly how they impede wound healing (Gottrup et al, 2013).

Patients with complex chronic wounds usually have comorbidities and other factors that may delay wound healing so the presence of a biofilm may not be the only reason that the wound does not heal (*Table 1*). Therefore, holistic assessment of the patient, optimising the patient's health, and identifying and treating other contributing factors that may affect the wound's ability to heal is of primary importance, as well as attempting to disrupt the biofilm.

Are biofilms an issue for clinicians?

Yes. Increasingly, clinicians are beginning to believe that biofilms play a key part in chronic non-healing wounds. Planktonic bacteria are usually removed or engulfed

by neutrophils; this process is called phagocytosis and occurs during the inflammatory phase of wound healing. It appears that the polysaccharide matrix secreted by a biofilm prevents this process from occurring, allowing the bacterial colony to establish itself within the wound. Once established, biofilms contribute to chronic inflammation within the wound in two ways:

- By releasing antigens that damage surrounding tissue
- By constantly releasing planktonic bacteria back into the wound environment, increasing neutrophils and macrophages into the wound bed, which secrete substances such as proteases into the wound, damaging surrounding tissue and further increasing the inflammatory response (Edwards and Harding, 2004). This prolonged inflammation leads to repetitive tissue damage, increased risk of infection and failure of the wound to progress to the next stage of healing called proliferation (Bryant and Nix, 2012).

Can you see a biofilm?

No. Identifying an infection in a wound can be difficult and can vary according to the signs and symptoms displayed (European Wound Management Association [EWMA], 2005) and the experience of the clinician undertaking the assessment (Dowsett, 2009).

Kingsley (2001) suggested a framework to assist clinicians in identifying wound infection known as the wound infection continuum. Within the continuum a wound may progress through a spectrum of states before overt clinical infection develops.

The first stage in the continuum is contamination. All wounds are known to be contaminated with bacteria, which do not always interfere with the healing process. When bacteria start to multiply, but still do not cause any issues with wound healing, this stage is known to be colonisation. Once the bacteria start to multiply and wound healing is impeded the wound reaches critical colonisation or local infection.

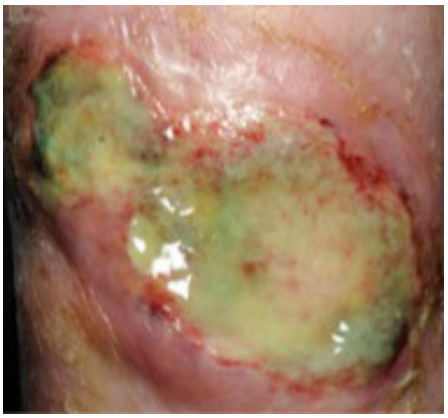

Figure 2. The wound contains a gelatinous material on the wound bed and has failed to respond to antibiotics.

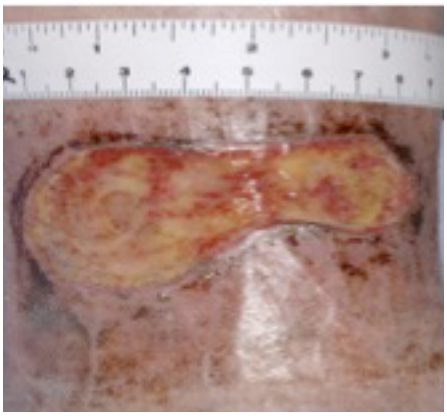

Figure 3. The wound has become static and non healing yet is not displaying obvious signs of wound infection.

Signs and symptoms of local infection/clinical infection have been described as an increase in serous exudate and inflammation, delayed healing, friable granulation, discoloured granulation pocketing in the wound base and wound breakdown (EWMA, 2005). Wounds then may go on to develop overt clinical infection where signs and symptoms are clearly visible. These signs and symptoms include oedema, erythema, pain increased purulent exudate and increased temperature (Gottrup et al, 2013).

There is some debate as to whether critical colonisation/local infection actually exists, as the characteristics are not definitive (EWMA 2005). However, it has been suggested that some of the signs and symptoms described are similar to wounds that may contain biofilms. These are wounds that do not display the characteristics of overt infection, but are failing to heal (Edwards and Harding, 2004). With no clearly defined criteria, a biofilm infection may not be easily recognised in clinical practice. Phillips et al (2010) suggested that the following characteristics may be present in a wound with a biofilm:

- Excessive exudate
- Poor quality granulation tissue
- Signs and symptoms of local infection
- Recurrent infection after stopping antibiotics
- Negative wound culture
- Infection lasting more than 30 days
- Gelatinous material that is easily removed form the wound surface (*Figure 2*)
- No healing despite optimal wound support and ensuring that any factors that may contribute to nonhealing have been addressed, e.g. treatment of comorbidities (*Figure 3*)
- Gelatinous surface reforms quickly.

Will a wound swab identify a biofilm infection?

No, wound swabs are not effective in identifying wounds that may have a biofilm present.

Unfortunately, standard clinical microbiology culture techniques for wound swabs are not able to identify biofilms. Wound swabs can be helpful in identifying planktonic

bacteria only and biofilms are currently only identified thorough advanced microscopy techniques (Keast et al, 2014).

Should I treat biofilms with an antibiotic or a topical antimicrobial?

No, antibiotics are not effective in treating biofilms. Antibiotics have been found to be ineffective in treating biofilms as they are unable to penetrate the extracellular matrix. This has been proved both *in vivo* and *in vitro* studies (Stewart and Costerton, 2001, Bjarnsholt et al, 2005). Antibiotics act on metabolically active bacteria and biofilms have developed a technique of hibernation where they cease to be metabolically active and this prevents the antibiotics from being effective (Edwards and Harding, 2004). Polymicrobial biofilms have also been shown to communicate and transfer genes, which further contributes to antibiotic resistance (Weigel et al, 2007). Indiscriminate treatment of biofilms with antibiotics is, therefore, not recommended and may contribute to bacterial resistance which is a worsening global issue (Gottrup et al, 2013).

What is the current strategy for treating biofilms?

There is currently no standardised strategy to treating biofilms. Recommendations advise a two-fold approach which includes

- Reduction of the biofilm bioburden
- Preventing reformation (Phillips et al, 2010).

Reduction of the biofilm bioburden can be done through wound bed preparation. This includes the physical removal of necrotic, devitalised and contaminated tissue, which provide the ideal environment for biofilm growth. The choice and method of debridement will depend on the clinicians' skill, knowledge and competency, as well as the availability of debriding agents, which may be costly.

Table 2. How to debride wounds that have a suspected biofilm.

Method of debridement	Considerations for use
Surgical debridement	Requires clinician with skill and competency Patient will need local anesthesia Increased risk of bleeding
Jet lavage — intense pressure irrigation with water	Equipment is costly Clinician needs skill and competency. Painful to the patient Local anesthesia may be required.
Bio-surgical (larvae)	May not be available due to cost Patient may not find the treatment palatable Offers fast debridement Larvae secrete substances which may reduce bacteria burden as well as debride devitalized tissue
Mechanical Monofilament cloth (e.g. Debrisoft®)	Relatively cost-effective Minimal training required May require frequent use
Or cleansing with an antiseptic solution that contains a surfactant e.g. polyhexanide PHMB with betaine (Prontosan®) or ocenidine with ethyhexyl glycereine (Octenili ®)	Dependent on local formulary availability Used as a cleansing agent and is applied to the wound bed Evidence indicates these agents may reduce surface tension of the wound and assist in removal of bacteria and devitalised tissue (Kaehn and Eberlein, 2009) Easy to use

Strohal et al (2013) advocate the methods of debridement shown in *Table 2*.

The process of debridement to remove biofilm colonies may need to be repeated several times over several weeks. A mature biofilm has been shown to recover from mechanical debridement within 24 hours (Costerton 1984). At present, there is no definitive time scale advocating how often or for how long the debridement process will need to be continued for, however, signs of wound improvement, decrease in slough and exudate may be a clinical indication that the biofilm has been removed (Philips et al, 2010)

Once the bioburden has been reduced, the prevention of reformation should be considered. This is where topical antimicrobial agents may be beneficial. The use of any topical antimicrobial agent will depend on availability of the product on local formulary and after holistic patient assessment. Other considerations before starting topical antimicrobial should be the condition of the wound bed, exudate levels, patient sensitivities and contraindications, and method of delivery.

Currently, there is no evidence to support the use of any one particular product as a first line treatment against biofilm reformation. Topical antimicrobials should, however, be selected on their ability to kill bacteria

rather than inhibiting growth (Phillips et al, 2010). Common antimicrobial agents, such as silver, honey, cadexomer iodine, PHMB and octenillin have been shown to be effective in treating planktonic bacteria. The evidence to support their effectiveness in mature biofilms is varied and more research is required – both *in vitro* and *in vivo* – to increase our understanding of which agents are the most effective against mature biofilms in clinical practice (Gottrup et al, 2013). However, these agents continue to assist in preventing planktonic bacteria from reforming and reattaching to the surface of the wound bed after debridement.

Conclusion

Biofilms are now thought to play an important role in chronic non-healing wounds and contribute to prolonging chronic inflammation within the wound. Treatment with antibiotics is considered ineffective and indiscriminate use may lead to further antibiotic resistance. The process of removing the biofilm by debridement and preventing reformation is currently promoted as the best approach to tackle biofilms.

Knowledge on the most appropriate debridement technique or most effective antimicrobial product to use is still in its infancy. However, until more research is undertaken into the eradication of biofilms, it appears that cleaning and using an antimicrobial is the most appropriate way of currently dealing with biofilms in clinical practice. WE

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