WOUND BIOFILMS: WHAT MAKES THEM STICK?

A biofilm is simply a community of microorganisms encased in a glue-like matrix that protects them from the environment. Many chronic, non-healing wounds fail to progress because of a biofilm being persistently present in the wound. This paper outlines the biology of biofilms and their relevance in the management of chronic wounds.

Bacteria usually exist in one of two states: planktonic, existing as free moving cell in the environment, or sessile, attached to a surface. Bacteria in the environment tend to form aggregates or colonies attached to the surface of non-living or living substrates. These aggregated cells secrete a glue-like substance primarily made of exopolysaccharide and proteins, called extracellular polymeric substance (EPS). The EPS completely encases them, offering protection from the external environment and forms a biofilm. The sessile bacterial cells within the matrix of the biofilm are characterised by their lack of movement (Costerton, 1999), whereas the planktonic cells are dispersed from the biofilm and colonise other areas.

Biofilms are commonly found in nature and disease (Costerton et al, 1987). Some examples are the biofilms found on rocks, pools and stagnant water, which can be seen as slime on the surface, and on teeth as dental plaque, which causes tooth decay, periodontal disease and gum disease.

It has been proposed that many chronic diseases seen in animals and humans are associated with biofilms, including some of the most common recurrent infections, for example, urinary tract infections, bacterial vaginosis, catheter infections and cystic fibrosis (Costerton et al, 1999).

The definition of a biofilm does vary between research groups, but an accepted definition is: “A biofilm is attached to a substrate and consists of many bacteria co-adhered by means of physical appendages and extra-cellular polymeric substances” (Garrett et al, 2008).

Formation of a biofilm
There are five key stages of biofilm development: initial attachment, irreversible attachment, maturation (two stages) and dispersion (Monroe, 2007).

The initial attachment is mediated through a weak chemical interaction between the surface involved and the outer envelope of the cell. The chemistry of the cell can vary considerably between species. It is important to note that not all microorganisms can form a biofilm, and it is also very dependent upon the chemistry of the surface involved.

Following this loose initial attachment, the bacterial cells anchor themselves permanently...
through more targeted attachment mechanisms, using their external structures such as the pili (also called fimbriae) or the capsule (Figure 1).

Once bacteria or fungi are attached to the surface, the overall surface chemistry may alter, and this may allow further bacterial species to adhere to either the surface or the other attached bacteria.

These cells eventually colonise the surface. During colonisation, the bacteria communicate with each other using chemical signals, producing EPS and allowing the biofilm to mature.

Once matured, the biofilm becomes tightly regulated via quorum sensing molecules. Other materials from the surrounding environment may be included in the EPS, including protein, DNA, minerals, blood components and fibrin, and these materials also influence the maturation of a biofilm.

The final stage of biofilm formation is dispersion. At this stage, the biofilm is established and may only change in shape and size (Munroe, 2007). In the dispersion stage, the biofilm releases planktonic cells to proliferate in other areas (Figure 2).

Biofilms can consist of a single species (for example, a Staphylococcus epidermidis biofilm in a shunt infection) or be formed from a mixture of bacterial and/or fungal species, which is termed a polymicrobial biofilm. A good example of a polymicrobial biofilm is dental plaque, where there are numerous species living in harmony within the EPS.

It is not possible to see biofilms with the naked eye or via standard microscopy, but they can be demonstrated in the laboratory using a variety of microscopic techniques, including scanning electron microscopy, epifluorescent microscopy and confocal scanning laser microscopy (Cooper, 2016). Electron microscopy is the most commonly used (Figure 3).

Using these microscopic techniques, it has been demonstrated that biofilms form irregular mushroom-like structures with fluid-filled channels between them, allowing moisture to be accessible to all

Figure 1. A bacterial cell showing pili and the capsule used for attachment.

Figure 2. The five stages of biofilm formation. Stage 1, initial attachment; stage 2, irreversible attachment; stage 3, maturation I; stage 4, maturation II; stage 5, dispersion.
areas of the biofilm (Monroe, 2007; Cooper, 2016).

**The importance of the EPS matrix**
The EPS is essential for survival of a biofilm. It provides the necessary architecture and micro-habitats to allow cells to cooperate and readily exchange genetic material (Cooper, 2016). The EPS protects the bacterial and/or fungal cells by creating further adhesion, retaining water and preventing access to phagocytes. In addition, encased enzymes can help digest complex molecules, providing extra nutrients when necessary and also offering protection against antimicrobial agents (Flemming and Wingender, 2010).

Sessile cells are more resilient to the environment. Researchers have shown that the growth rate of cells within the biofilm, especially at the cell/surface interface, is reduced compared to cells near the upper area of the biofilm. This has an impact on antimicrobial susceptibility because the mode of action of many antibiotics (for example, erythromycin and tetracycline) relies on rapidly growing cells.

Studies have shown that the minimum inhibitory concentration for antibiotics can be up to 1,000-fold higher than those required to inhibit planktonic cells (Ceri et al, 1999; Olson et al, 2002; Qi et al, 2016). Generally a higher concentration of the antimicrobial agent (such as antibiotics, disinfectants and antiseptics) will be needed in order to have an effect on a biofilm, compared to bacteria in the planktonic state (Stewart and Costerton, 2001; Bridier et al, 2013).

**Biofilms in wounds**
The association of biofilms with chronic wounds was demonstrated by James et al (2008), and has since developed into a credible cause for wound chronicity and delayed wound healing (Wolcott et al, 2008).

A number of studies have demonstrated the presence of biofilms in chronic wounds. A recent meta-analysis and systematic review of nine studies showed that biofilms were present in over 78% of wounds (Malone et al, 2017). However, biofilms have only been shown to be present in 6% of acute wounds (James et al, 2008).

Most acute wounds heal without consequence if the patient’s immune system is not compromised. However, if there is some physiological problem, such as poor vascular supply, diabetes or arterial disease, then wound healing can be delayed, leaving the wound open longer to bacterial contamination, colonisation and ultimately biofilm formation. If a biofilm becomes resident in the wound bed, then it can cause stimulation of the inflammatory response, resulting in increased neutrophils and macrophages, matrix metalloproteases, reactive oxygen species and elastase in the wound bed (Manavathu et al, 2014).

This cycle of inflammatory response and colonisation with a polymicrobial biofilm needs to broken before a chronic wound will start to heal.

**Managing biofilms**
A treatment pathway needs to be initiated by the clinician. Firstly, if the wound looks clinically infected, a swab should be taken to ascertain if there are any pathogens present that could be causing the overt symptoms. Empirical antibiotic therapy should be given while awaiting the results from the laboratory. The antibiotics should prevent the spread of the pathogen from the wound and reduce the risk of sepsis.

A position statement on antimicrobial stewardship in wound care recommended that once results were available, then a narrow spectrum antimicrobial agent should be administered for the minimum time period to help reduce the development of antimicrobial resistance (Lipsky et al, 2016).

Antimicrobial stewardship guidelines are available in primary care and hospital trusts, and they should be referred to when treating patients with problematic wounds. The National Institute for Health and Care Excellence (NICE) has produced...
guidelines and quality standards on antimicrobial stewardship (NICE, 2015, 2016).

If the wound does not look clinically infected, but is static and not healing, it is highly likely that a polymicrobial biofilm is present (Malone et al, 2017). This polymicrobial biofilm may contain known pathogen(s), and they may be contributing to the perpetual cycle of inflammation seen in a chronic wound without causing overt infection.

In order to stop this cycle of an inflammatory, non-healing wound, reducing bioburden and disrupting the tightly regulated biofilm may allow healing. A series of cleansing and/or debridement and application of a topical antimicrobial agent (antiseptic) cycles is therefore required (Philips et al, 2010a; 2010b; Wolcott et al, 2009; 2010). The evidence for this management comes from in vitro and in vivo studies.

Laboratory studies have shown that planktonic bacteria attach within minutes to a surface and form microcolonies, and within 2–4 hours begin to form the EPS, forming a fully mature biofilm within 24–48 hours, depending upon the species and growth conditions (Philips et al, 2010a).

In laboratory studies where a mature biofilm was disrupted by sharp debridement, it was shown that the biofilm rapidly reformed following this disruption and there was a therapeutic window when topical antimicrobial agents could be applied to prevent the biofilm reforming (Philips et al, 2010b). Wolcott and Rhoads (2008) established a 77% healing rate in patients with critical limb ischaemia and proposed biofilm-based wound care management using cleansing, debridement and application of topical antimicrobial agents.

A number of biofilm-based wound care best practice statements have been produced (World Union of Wound Healing Societies, 2016; Wounds UK, 2017). A decision tree should be used in the treatment pathway (Figure 4). If there are no signs of improvement in the wound, then the topical antimicrobial agents should be reviewed and changed as appropriate.

The person undertaking the wound care may not be qualified to undertake some of the more aggressive debridement procedures (such as surgical debridement and hydrosurgery). In such cases, the patient should be referred to a more qualified practitioner, such as a tissue viability nurse or podiatrist.

Biofilm-based wound care aims to reduce bioburden through disruption by cleansing and debridement, and to prevent biofilm reformation by application of a topical antimicrobial agent.

The best combination of cleansing agent, debridement technique and topical antiseptic has yet to be established for all wound types, but a number of new and existing products in each category are being tested in vitro for their ability to reduce or disrupt biofilm and prevent formation (Rhoads et al, 2008; Wolcott et al, 2009; 2010).

**Conclusion**

Biofilms must be considered as the cause of most non-healing chronic wounds if the patient’s general health is not the primary issue.
medical condition is good and there are no obvious other issues. It is important that regular cleansing and debridement is used to insult the EPS and biofilm in order to create a therapeutic window when topical antimicrobial agents can be applied.

Systemic therapy should not be used if there are no systemic signs of spreading infection.

References


