Surfactants and their role in biofilm management in chronic wounds

The use of surfactants in wound care represents an emerging treatment for complicated or chronic wounds, due to their potential ability to prevent and breakdown recalcitrant biofilms. Biofilms are present in all chronic wounds and have been reported to prevent wound closure by contributing to chronic inflammation and increasing a wounds propensity to infection, hence, their removal is significant to timely wound healing. Although the evidence demonstrating the effectiveness of surfactants is limited primarily to *in vitro* studies, there are a growing number of clinical studies demonstrating their ability to prevent biofilm formation and, consequently, enhance wound healing.

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Chronic wounds are defined as wounds that show no signs of healing after 4–6 weeks of treatment and can form in various scenarios, such as ulcers, amputations and transplants (Paavola et al, 2000; Clark, 2004; Wolcott and Dowd, 2011). All chronic wounds are a significant problem in healthcare and are estimated to affect 1–2% of the population in the developed world at some point during their lifetime (Gottrup, 2004).

Chronic, as well as acute wounds, represent ideal environments for the formation of biofilms (Percival et al, 2012). Biofilms persist in chronic wounds causing prolonged inflammation and, consequently, delayed healing, and increase a wound propensity to infection (Percival et al, 2017a). Several studies have demonstrated the evidence of biofilms in a variety of chronic wounds. For example, in the study by Martinez-Velasco et al (2014), biofilms were visualised in all 20 chronic wounds examined and Honorato-Sampaio et al (2014) confirmed the presence of biofilms in all 45 venous leg ulcers that were inspected. These recent studies have helped to validate the hypothesis proposed by Percival et al (2012) that all chronic wounds contain biofilms.

Biofilms are difficult to manage and treat as the microorganisms present in the sessile state have an increased tolerance to both antimicrobials and the immune system (Stewart and Costerton, 2001; Percival et al, 2012). Consequently, new technologies and interventions are required to assist in effective biofilm management.

Surfactants are thought to play a role in biofilm management by aiding in the debridement of wounds, helping to remove devitalised tissue and slough (Mayer et al, 2018) and also removing the biofilm itself (Salisbury et al, 2018), consequently reducing chronic inflammation and promoting wound healing (Yang et al, 2017; Salisbury et al, 2018). This brief review will explore the literature on some commonly used surfactants and their role in biofilm management in the context of chronic wounds.

Biofilm formation

Microorganisms exist in two phenotypic states in the environment, planktonic (free-floating) and sessile (attached to surfaces or other microbes). Microorganisms in biofilms differ from free-floating microbes in their phenotypic traits, gene expression, antibiotic recalcitrance and host interaction (Whiteley et al, 2001; Zhao et al, 2013). Biofilms are communities of sessile microorganisms and account for 99.9% of the bacteria present in the environment (Donlan and Costerton, 2002). A biofilm consists of microorganisms encased in a matrix of extracellular polymeric substance (EPS) which is composed of water, polysaccharides, nucleic acids (extracellular DNA) and proteins (Donlan and Costerton, 2002). Biofilms initially form when microbes weakly attach to a surface, or each other, and then subsequently attach strongly (Joshua et al, 2006). Following this, they are able to form aggregates (both on a surface and within fluids) and grow using chemotaxis and quorum sensing mechanisms (Tomaras et al, 2003).

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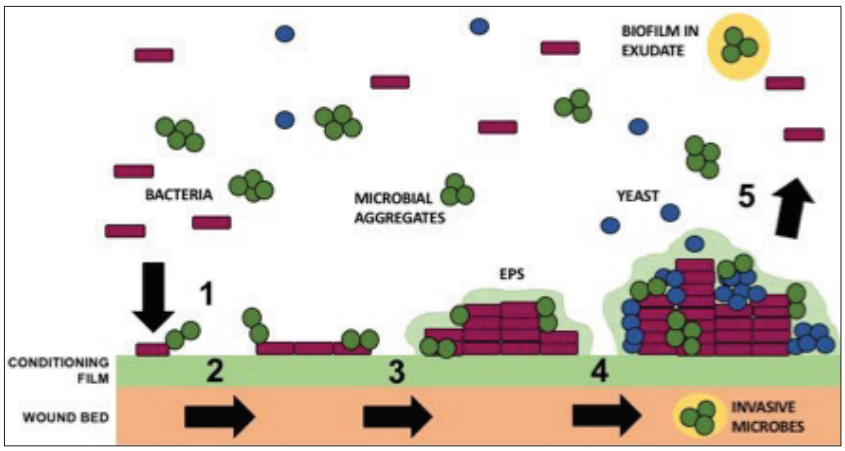

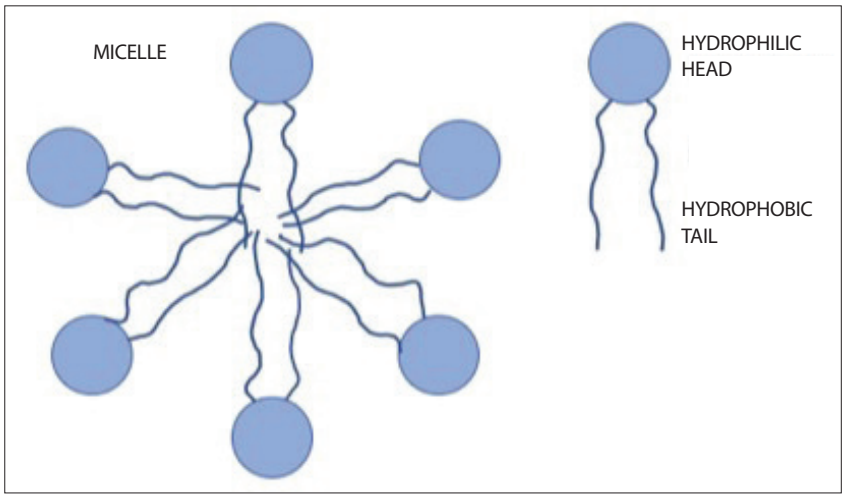

Figure 1 (top). Formation of a biofilm in a wound bed. 1. Microrganisms attach to the conditioning film formed on the wound bed. 2. Formation of a microbial aggregates. 3. Multi-layer biofilm and EPS formation. 4. Mature multispecies biofilm (on wound bed and on the wound surface and also in wound exudate (Percival, 2018). 5. Detachment and dissemination of microbes.

Figure 2 (above). Structure of a surfactant molecule and the formation of a micelle.

A mature biofilm consists of aggregated microorganisms encased in EPS, as shown in *Figure 1*.

Wound biofilms

The presence of biofilms in wounds can reduce healing rates and increase the chances of infection leading to the formation of chronic wounds (Percival et al, 2017a). Historically, biofilms were reported to be responsible for 65% of the bacterial infections present in chronic wounds (Sanderson et al, 2006). However, in the authors' opinion it is hypothesised that all chronic wounds will contain biofilms, which reside within either or both the benign or pathogenic states (Percival et al, 2012; Honorato-Sampaio et al, 2014). In addition, recent evidence has shown that biofilms exist in at least five locations in wounds: within the wound bed, within deep tissue, within and on slough and necrotic tissue, and also on wound dressings (Percival et al, 2017b). Consequently, the management of biofilms within a wound should not be based on one universal approach, but necessitates the employment of a multifaceted methodology centred on a personalised strategy for each individual patient.

The necrotic tissue and slough present in chronic wounds provide ideal surfaces for microbes to adhere to, thus facilitating biofilm formation (Wolcott et al, 2008). Biofilms are able to form rapidly in wounds, as demonstrated by (Kennedy et al, 2010) who visualised biofilm formation in burn wounds 7–31 days post injury (Nakagami et al, 2008) and who identified signs of early biofilm formation in infected wounds 3–7 days post injury.

Chronic wounds caused by biofilms are persistent, since sessile microbes are capable of survival in concentrations of antibiotics 100–1,000 times greater than their planktonic equivalents (Stewart and Costerton, 2001). Sessile microbes have a higher tolerance to antimicrobials due to poor antibiotic penetration, phenotypic changes and the formation of persistent cells (Stewart, 2002). It was initially thought that the EPS was primarily responsible for this increased recalcitrance, as it limits the diffusion of antimicrobials, thus resulting in sub-therapeutic levels reaching the microbes. However, it has been suggested that other mechanisms may also be involved (Anderl et al, 2003). For example, the phenotypic switch from the metabolically active state of planktonic microbes to the more dormant state observed in sessile microbes, may prevent the antibiotic's ability to kill the microorganisms, since many rely on disrupting metabolic processes (Nguyen et al, 2011).

Biofilms have also been shown to cause chronic inflammation in wounds, as the elevated levels of cytokines produced by macrophages in response to the biofilm, lead to an increased recruitment of immune cells (Yager and Nwomeh, 1999). This causes the over-production of proteases and reactive oxygen species, which break down the proteins involved in the wound-healing process (Mast and Schultz, 1996). There has been evidence to suggest that the EPS contributes greatly to the issue of chronic inflammation. For instance, Seth et al (2012) observed that EPS-deficient *Pseudomonas aeruginosa* did not delay healing in ischaemic rabbit-ear wounds.

Treatment of chronic wound biofilms

In order to eliminate this issue of chronic inflammation, both the microbial component of the biofilm and the EPS must be removed. The removal and breakdown of the EPS is as important as killing the microorganisms as it contributes greatly to the inflammatory effects of the biofilm and also plays a protective role by shielding the microbes from antimicrobial agents, including antibiotics (Hemmi et al, 2001; Flemming and Wingender, 2010). 'Debridement' is the term used to describe the removal of dead and damaged

Table 1. Antibiofilm activity of various biosurfactants.

Biosurfactant	Source	Effect	Reference
Rhamnolipids	*P. aeruginosa*	Reduction in biofilm formation of *Candida spp*	Dusane et al, 2012
Fengycin	*Bacillus subtilis*	Inhibition of biofilm formation of *Escherichia coli* and *Salmonella enterica*	Rivardo et al, 2009
Glycolipid	*Serratia marcescens*	Anti-biofilm activity against *Candida albicans* and *P. aeruginosa*	Dusane et al, 2011
Lipopeptide	*Bacillus circulans*	Anti-biofilm activities against *E. coli, Proteus vulgaris, Salmonella typhimurium*	Das et al, 2009

tissue, which constitutes a supportive structure for biofilms, from a wound. Sharp, or surgical debridement, is the most popular technique and is often performed when safe to do so. This involves the use of a sterile scalpel or scissors to physically remove tissue (Schultz et al, 2017). However, this is not always safe, hence debridement and removal of slough (desloughing) using surfactants represents a potential treatment for chronic wound cleaning and biofilm removal (Percival et al, 2017b; Yang et al, 2017).

Surfactants

Surfactants have various roles in wound care, including wound cleansing and biofilm management (Yang et al, 2017). Autolytic debridement is a useful alternative if the clinician does not possess the skills to perform surgical debridement, and also helps to reduce the pain and increased costs associated with surgical procedures (Jovanovic et al, 2012; Malone and Swanson, 2017). Surfactants are surface active agents that lower the surface tension between two liquids, a gas and a liquid or between a liquid and a solid (Banat et al, 2000). They are able to do this by forming structures called micelles that consist of a hydrophobic tail and hydrophilic head *[Figure 2]*. This increases the wettability of the surface and solubility of materials that would otherwise not dissolve into each other (Banat et al, 2000).

Surfactant classification

Surfactants can be categorised according to their behaviour in aqueous solution, in which each category is defined according to the charge on the hydrophilic head of the surfactant molecule (Kume et al, 2008). Cationic surfactants have a positive charge, anionic have a negative charge, non-ionic are uncharged and amphoteric surfactants have a positive and negative charge at intermediate pH (Kume et al, 2008). Surfactants can also be separated into two groups, synthetic and natural (Percival et al, 2017b).

Biosurfactants

Biosurfactants are non-ionic, natural surfactants produced by a wide variety of organisms and are able to prevent bacterial attachment and, consequently, biofilm formation by altering cell surface characteristics within the biofilm matrix (Banat et al, 2014; Coronel-Leon et al, 2016). It has been suggested that biosurfactants may be favoured in some cases over synthetic surfactants due to their biodegradability and low toxicity (Dusane et al, 2011). The anti-biofilm activity of a range of biosurfactants has been investigated in various studies *[Table 1]*.

Although the anti-biofilm activity of biosurfactants is limited to mainly *in vitro* studies, Piljac et al (2008) observed that rhamnolipids were able to improve the healing of a decubitus ulcer in a clinical study. Hence, biosurfactants may have an ability to prevent biofilm formation in the context of wound care (Banat et al, 2014).

Synthetic surfactants

There are several examples of synthetic surfactants in wound care with the most well-researched being poloxamers and betaines. Poloxamers are non-ionic, synthetic surfactants composed on a central hydrophobic chain of polyoxypropylene and two hydrophilic chains of poloxyethylene. The chain length can be adjusted to produce different types of poloxamers (Baskaran et al, 2001). Poloxomer 188 is noted to have an inhibitory effect on biofilm formation in *ex vivo* porcine skin with either *Staphylococcus aureus* or *Acinetobacter baumannii* persisting in the wound following treatment (Yang et al, 2018). Plurogel® (Medline Industries Inc) is an example of a wound gel containing the surfactant Poloxamer 188, which has shown its capability in reducing the inflammatory effects caused by biofilms by modulating the secretion of pro-inflammatory cytokines (Salisbury et al, 2018). Additionally, Poloxamer 407 has been reported to reduce biofilm formation by disrupting the attachment of *Staphylococcus* epidermidis to the wound surface (Romic et al, 2016). Consequently, poloxamers, in particular, represent potential effective surfactants capable of managing wound biofilms to promote faster healing.

Combining surfactants and antimicrobials

In order to further enhance the effects of surfactants on biofilm removal, they can be combined with antimicrobials. For example, the surfactant Poloxomer 188 has been combined with

the antimicrobial silver sulfadiazine (SSD), and has been shown to eliminate all viable bacteria from the skin within 3 days of treatment application (Yang et al, 2017). Additionally, the use of the synthetic surfactant undecylenamidopropyl betaine and the antimicrobial polyhexanide, has been shown to result in dramatic improvements in wound healing in 7 out of 10 patients within 3 weeks, in addition to biofilm elimination (Horrocks, 2006). The combination of 0.1% polyhexanide and 0.1% betaine was also used in an additional study investigating its ability to manage infected wounds, and a 5.3-log to 5.8-log reduction in the presence of *S. epidermidis, P. aeruginosa, C. albicans, S aureus, Enterococcus faecalis, E. coli* and several other strains commonly found in wound biofilms was observed (Minnich et al, 2012).

Surfactants in biofilm management

Surfactants are able to disrupt biofilms and treat infection by various mechanisms. They can be left *in situ* for approximately 15 minutes to help loosen necrotic tissue or be scrubbed lightly on the wound surface with a sterile gauze (Malone and Swanson, 2017). The micelles formed by the surfactant are able to change from a collapsed to an expanded state allowing wound debris to become trapped. This creates a rinsing action allowing the wound to be cleaned and preventing microbes from adhering to the wound surface, thus preventing biofilm formation (Percival et al, 2017b).

This rinsing action is also effective in removing older biofilms from the wound surface by disrupting the EPS, allowing the microbes to become more susceptible to the host's immune response and antibiotics (Zhao et al, 2013; Percival et al, 2017b). In addition, to the disruptive effects of surfactants on the biofilm's structure, they can also be used as carriers for antimicrobials. Gel-based surfactants allow the antimicrobials to stay localised to the wound as they are more adhesive than liquids, hence, they allow the sustained delivery of antimicrobials to target the biofilm (Zölß and Cech, 2016).

Conclusions

The efficacy of surfactants in biofilm management has been well documented *in vitro* and there are now a growing number of studies highlighting the beneficial effects in clinical scenarios. It has been observed that both synthetic and natural surfactants have an ability to remove and prevent biofilms (Piljac et al, 2008; Yang et al, 2017). The evidence demonstrating the success of surfactants in wound biofilm management favours the use of synthetic surfactants, such as poloxomers and betaines, which appear to be a promising solution to prevent and treat chronic wounds (Horrocks, 2006; Percival, 2018). The use of surfactants to promote debridement and desloughing by cleansing the wound provides an alternative to the current gold-standard of sharp debridement, which is associated with increased pain, costs and the requirement for a skilled clinician (Malone and Swanson, 2017). Overall, the use of surfactant-based wound dressings is an emerging treatment with a proven ability to prevent and treat chronic wounds caused by biofilm formation. WINT

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